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LEVELS OF CONTAMINANTS IN BIVALVE MOLLUSCS OF BRITISH COLUMBIA

Regional Program Report No: 83-19

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DEPARTMENT OF ENVIRONMENT
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LEVELS OF CONTAMINANTS IN BIVALVE MOLLUSCS OF BRITISH COLUMBIA

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ABSTRACT

This report summarizes and reviews British Columbia data for specific contaminants in bivalves (Phylum Mollusca, Class Pelecypoda or Bivalvia). This report concentrates on those contaminants associated with anthropogenic activities and is restricted by the nature of the data to a discussion of heavy metals, PCBs and PAH.

Studies conducted in B.C. and elsewhere in the world related to the use of bivalves as indicator organisms and the effects of toxic chemicals on bivalves are reviewed.

The review of British Columbia data shows high levels of some contaminants have been reported in some shellfish. These high levels were associated with known pollution sources or spills and did not occur in major commercially or recreationally harvested shellfish areas.

RÉSUMÉ

Ce rapport résume et étudie les données recueillies en Colombia-Britannique sur certains contaminants que l'on retrouve dans les bivalves (mollusques de la lignée des phylums et de la classe des Pélécypodes). Le rapport traite surtout des contaminants associés aux activités anthropogènes. Etant donné la nature des données étudiées, le rapport ne porte que sur les métaux lourds, le PCB et le PAH.

Le rapport fait état des études menées en Colombie-Britannique, et ailleurs dans le monde, sur l'utilisation des bivalves comme organismes indicateurs ainsi que sur les effets d'agents chimiques toxiques sur les bivalves.

L'étude des données recueillies en Colombie-Britannique révèle le présence de grandes quantités de contaminants dans certains doquillages. Cette contamination, qui est causée par des agents de pollution de source connue ou par des produits toxiques déversés dans les eaux, n'atteint pas les principales zones de récolte commerciale ou sportive de coquillages.

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LEVELS OF CONTAMINANTS IN BIVALVE MOLLUSCS OF BRITISH COLUMBIA

1 INTRODUCTION

The oceans have always been the repository for much of the wastes generated on earth. Among these wastes is an ever-increasing quantity of toxic materials.

The awareness of dangers from biological wastes in natural waters, particularly in the spread of disease, is of comparatively long standing. Over fifty years ago, bacteriological monitoring of shellfish-growing waters was adopted in the United States as the U.S. National Shellfish Sanitation Program (1), in response to a typhoid epidemic from contaminated oysters. A similar shellfish sanitation program was subsequently adopted by the Canadian Department of Health and Welfare, and is carried out by the Environmental Protection Service of Environment Canada, and Fisheries and Oceans Canada.

Unlike bacteria, chemical and organo-chemical contaminants (of bivalves molluscs, eg. oysters, clams, mussels) have not received serious attention until the last decade. Such contaminants include heavy metals and a growing number of organochemical products and by-products (2, 3). The latter include a variety of pesticides, polychlorinated biphenyls (PCBs), benzenes, chlorinated phenols and pthalate esters. Polycyclic aromatic hydrocarbons (PAH), some of which are known carcinogens, are also being monitored in the marine environment. Table 1 lists compounds which have been identified by the U.S. Environmental Protection Agency as unambiguous priority pollutants.

The contribution of heavy metals to the environment is increased substantially through man's mining activities as shown in Table 2 (2). The mobilisation rate for most metals is several fold higher than that achieved by geological weathering. Some of these wastes are stored indefinitely in marine sediments while others are recycled through chemical and biological processes (5).

TABLE 1: U.S ENVIRONMENTAL PROTECTION AGENCY PRIORITY POLLUTANTS

Metals

Antimony
Arsenic
Beryllium
Cadmium
Chromium
Copper
Lead
Mercury
Nickel
Selenium
Silver
Thallium
Zinc

Miscellaneous

Cyanide (mg/l)
Phenolics (AAP)
Asbestos

Pesticides

Aldrin
Chlordane
Dieldrin
4, 4'DDT
4, 4'DDE
4, 4'DDD
Alpha Endosulfan
Beta Endosulfan
Endosulfan Sulfate
Endrin
Endrin Aldehyde
Heptachlor
Heptachlor Epoxide
Alpha BHC
Beta BHC
Gamma BHC (Lindane)
Delta BHC
Toxaphene
PCB 1016
PCB 1221
PCB 1232
PCB 1242
PCB 1248
PCB 1254
PCB 1260

Base/Neutral Extractibles

Acenaphthene
Benzidine
1, 2, 4-Trichlorobenzene
Hexachlorobenzene
Hexachloroethane
Bis(2-Chloroethyl) Ether
2-Chloronaphthalene
1, 2-Dichlorobenzene
1, 3-Dichlorobenzene
1, 4-Dichlorobenzene
3, 3-Dichlorobenzidine
2, 4-Dinitrotoluene
2, 6-Dinitrotoluene
1, 2-Diphenylhydrazine
Fluoranthene
4-Chlorophenyl Phenyl Ether
4-Bromophenyl Phenyl Ether
Bis(2-Chloroisopropyl)Ether
Bis(2-Chloroethoxy)Methane
Hexachlorobutadiene
Hexachlorocyclopentadiene
Isophorone
Naphthalene
Nitrobenzene
N-Nitrosodimethylamine
N-Nitrosodi-N-Propylamine
N-Nitrosodiphenylamine
Bis(2-Ethylhexyl) Phthalate
N-Butyl Benzyl Phthalate
Di-N-Butyl Phthalate
Di-N-Octyl Phthalate
Diethyl Phthalate
Dimethyl Phthalate
Benzo(A)Anthracene
Benzo(A)Pyrene
Benzo(B)Fluoranthene
Benzo(K)Fluoranthene
Chrysene
Acenaphthylene
Anthracene
Benzo (GHI) Perylene
Fluorene
Phenanthrene
1, 2, 5, 6-Dibenzanthracene
Indeno (1, 2, 3-CD)Pyrene
Pyrene
TCDD

TABLE 1: U.S. ENVIRONMENTAL PROTECTION AGENCY PRIORITY POLLUTANTS

(Continued)

Acid Extractibles

2, 4, 6-Trichlorophenol
P-Chloro-M-Cresol
2-Chlorophenol
2, 4-Dichlorophenol
2, 4-Dimethylphenol
2-Nitrophenol
4-Nitrophenol
2, 4-Dinitrophenol
4, 6-Dinitro-O-Cresol
Pentachlorophenol
Phenol

Volatile Organics

Acrolein
Acrylonitrile
Benzene
Carbon Tetrachloride
Chlorobenzene
1, 2-Dichloroethane
1, 1, 1-Trichloroethane
1, 1-Dichloroethane
1, 1, 2-Trichloroethane

Volatile Organics (cont.)

1, 1, 2, 2-Tetrachloroethane
Chloroethane
Chloroform
1, 1-Dichloroethylene
1, 2-Trans-Dichloroethylene
1, 2-Dichloropropane
CIS-1, 3-Dichloropropene
Trans-1, 3-Dichloropropene
Ethylbenzene
Methylene Chloride
Methyl Chloride
Methyl Bromide
Bromoform
Bromodichloromethane
Trichlorofluoromethane
Dichlorodifluoromethane
Dibromochloromethane
Tetrachloroethlene
Toluene
Trichloroethylene
Vinyl Chloride
Bis(Chloromethyl) Ether
2-Chloroethyl Vinyl Ether

TABLE 2: THE EFFECTS OF MAN ON THE MOBILISATION RATES OF TRACE METALS [GEOLOGICAL RATES OF METAL MOBILISATION ARE COMPUTED FROM ANNUAL RIVER DISCHARGES TO OCEANS (2)]

ELEMENT	GEOLOGICAL RATE (10^3 metric tons/year)	MAN-INDUCED RATE (mining) (10^3 metric tons/year)	TOTAL IN OCEANS (10^6 metric tons)
Iron	25 000	395 000	4 110
Manganese	440	8 150	2 740
Copper	375	6 000	4 110
Zinc	370	5 320	6 850
Nickel	300	481	2 740
Lead	180	3 200	41
Molybdenum	13	74	13 700
Silver	5	9	137
Mercury	3	10.5	68
Tin	1.5	227	14
Antimony	1.3	65	274
Cadmium	No data	17	68

While there is still little evidence of anthropogenic contaminants in the open ocean (3, 6), coastal waters and estuaries are feeling the brunt of human activity (7). It is only in recent years we have come to realize that toxic compounds cannot be flushed into the sea with impunity. The sobering evidence of the Minimata tragedy created a worldwide awareness and concern about the disposal of toxic heavy metals into the marine environment (8). The resultant flurry of research into the fate of mercury and other metals in the coastal waters has produced much evidence attesting to the bioaccumulation of toxic compounds by marine organisms, particularly bivalves (2, 9). As many studies have shown, the growing waters for oysters, clams and mussels are also the receiving waters for a broad spectrum of anthropogenic pollutants. Shellfish have been discovered to bioaccumulate not only metals but also organochemicals (10, 11, 12) as well as radionuclides (4, 13).

In British Columbia, federal and provincial government agencies, industries and individual scientists have undertaken numerous studies since the early 1970's related to pollution in Georgia Strait, reviewed by Parsons in 1972 (14) and Waldichuk in 1983 (15). Invertebrates, including many species of molluscan bivalves, have been a part of these studies. However, no specific investigation has correlated toxic pollutants with commercially important shellfish.

The harvest areas for commercial molluscan shellfish in B.C. are shown in Figure 1. The Environmental Protection Service and Department of Fisheries and Oceans regularly monitor the areas for contamination by fecal coliforms and paralytic shellfish poison (PSP). Some tests for heavy metals are made by the Fish Inspection Laboratory, Dept. of Fisheries and Oceans, on shellfish for export and occasionally by the Environmental Protection Service on environmental samples. However, these tests are not conducted specifically on B.C. products and the bacteriological monitoring programme has no equivalent for chemical pollutants in B.C. waters.

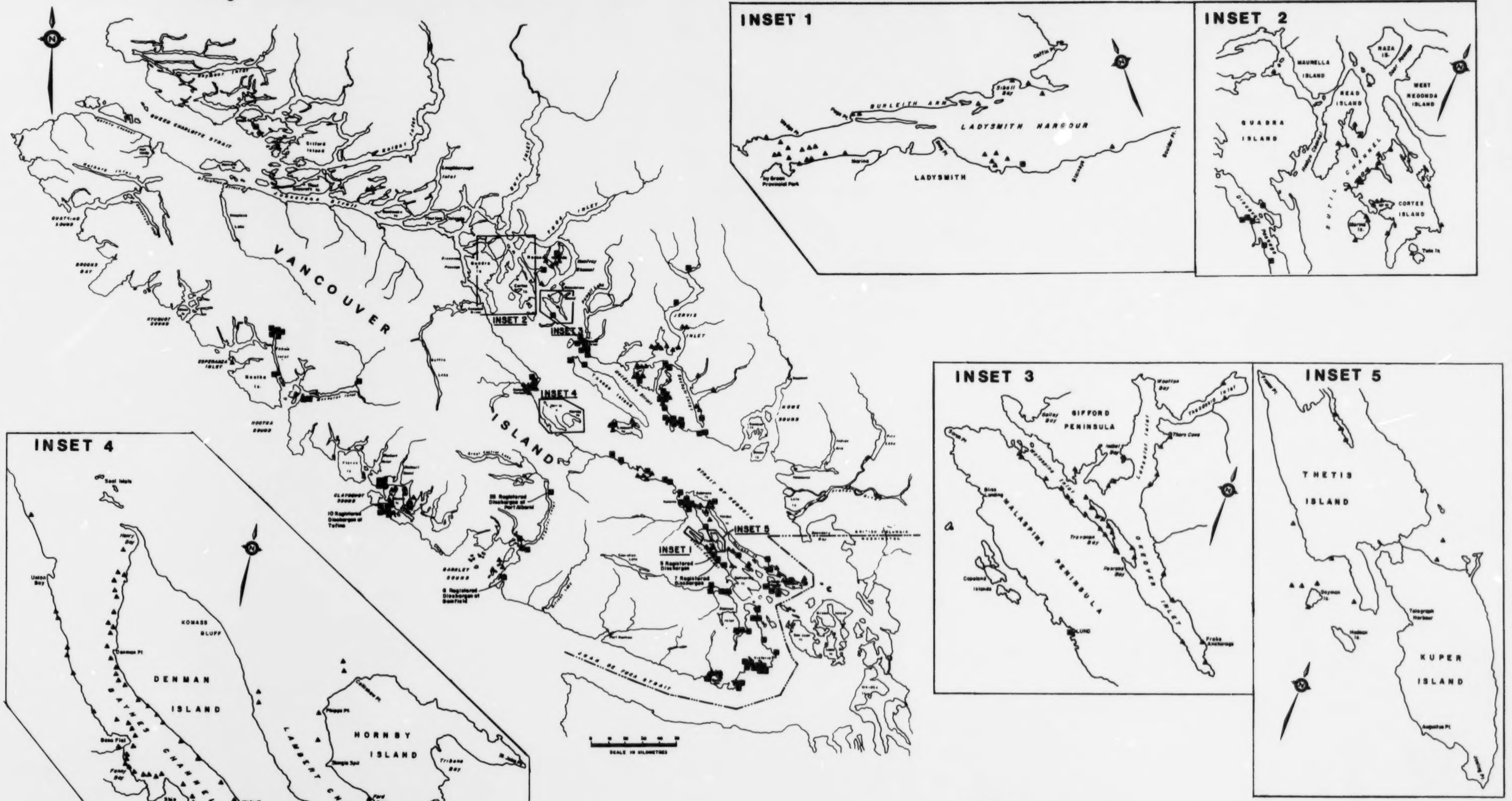

FIGURE 1

COMMERCIAL SHELLFISH HARVESTING AREAS IN RELATION TO IDENTIFIED POLLUTION SOURCES IN BRITISH COLUMBIA

Besides concern about acute toxic effects from chemical contaminants in shellfish, there is a growing concern about sub-lethal, chronic effects on humans, shellfish and other marine organisms. In this report bivalves are considered (1) as an important natural resource; (2) as vectors of potentially dangerous compounds; and (3) as indicators of chemical pollution in the marine environment. Heavy metals of particular concern in B.C. and elsewhere are mercury, lead, cadmium, zinc, and to a lesser degree copper, silver and arsenic. PCBs have been identified in vertebrates and invertebrates in the Fraser River Estuary. Benzo(a)pyrene, a carcinogenic PAH, has been the subject of studies in relation to B.C. bivalves. Pesticides, chlorinated phenols and other organo-chemicals have been identified in low concentrations in the Fraser River Estuary (16) however the data is extremely limited for shellfish and is not reported herein. Radionuclides, considered a contaminant in coastal waters in many parts of the world (17), have not been identified in B.C., and are also not included.

This report presents (in Section 3) the results of studies on contamination of B.C. bivalves by heavy metals, PCBs and PAH. Section 2 provides background information about these contaminants in general, with special reference to bivalves as indicator organisms. Greater attention is given to heavy (trace) metals, not only because more information is available, but also because many metals are especially dangerous to humans and other organisms in excessive amounts. Three such metals, mercury (18), cadmium (19) and lead (20), are the subjects of special reports by Environment Canada. Appendix VII includes methods of field sampling, tissue preparation and analytical methods for the various toxic compounds. The conclusions attempt to integrate results of research elsewhere to the situation of toxic chemical pollution, actual or potential, of marine waters in British Columbia.

2 A REVIEW OF SELECTED TOXIC CONTAMINANTS

2.1 Heavy Metals

Heavy metals require a simple definition. They can be defined by their position in the Periodic Table, as outlined in the block in Table 3 (21), or as having a density greater than 5 (22). Trace metals, another term for heavy metals, refers to their presence in concentrations less than 400 parts per million (ppm)(22). As insoluble compounds in minerals, metals are, for the most part, harmless; but their soluble derivatives may be toxic. They enter the environment by natural weathering of rocks and leaching of soils and vegetation, by volcanic activity and forest fires. Man contributes metals to the environment by mining and smelting activities, by combustion of fossil fuels and by processing, manufacturing and industrial waste disposal.

Most of the metal load is transported by water in dissolved or particulate state, and much of it reaches the ocean in rivers and streams or general land run-off. Metal in sediments may be reduced or oxidized by microbes and re-released to the overlying water (23).

2.1.1 Heavy Metals in Marine and Estuarine Environments.

Metals in an aquatic environment may exist in dissolved or particulate forms. They may be dissolved as (a) free hydrated ions or (b) as complex ions, chelated with inorganic ligands (e.g. OH^-, CO_3^{2-}, Cl^-), or with organic ligands such as amines, humic and fulvic acids and proteins. Particulate metal forms may be found as; (a) colloids or aggregates (e.g. hydrated oxides); (b) adsorbed on different types of particles; (c) precipitated as metal coatings or particles; (d) incorporated into organic particles such as plankton; (e) held in the structural lattice in crystalline detrital particles (23). The physical and chemical forms of metals in water are controlled by environmental variables such as pH, redox potential, ionic strength, salinity, alkalinity, presence of organic and particulate matter, biological activity and the intrinsic properties of the

TABLE 3: HEAVY METALS (21)

H																He
Li	Be										B	C	N	O	F	Ne
Na	Mg										Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac														

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lw

metal. Changes in these variables result in transformation of the chemical form and contribute to the availability, accumulation and toxicity to aquatic organisms (23).

In coastal waters, the concentrations of heavy metals decrease with distance from river mouths. The diminution is not caused by dilution alone, but by loss to the sediments and to biota. In the marine environment the increase in salinity, away from river mouths, can result in a salting out of large molecular weight fractions and flocculation of inorganic matter, producing increased particle sizes, so that incorporated metals end up in the sediments. On the other hand, metals attached to particles may be mobilized from the carrier particles by chlorine ions, and become available to the biota (23).

The water quality study in the Fraser River Estuary (24) has investigated the metal load, its sources, components and fate in the Fraser River System (25). Information from the study related to bivalves is included in Section 3.5. Heavy metals and related trace elements in natural waters have been reviewed by Leland, et al. (26). A number of studies have investigated metal pollution in coastal waters and estuaries specifically (26-30), while others have dealt with the general flow and mechanism of transport of metals (31, 32).

2.1.2 Heavy Metals in Living Systems. Although the focus of this report is on metals as compounds which may be toxic to humans, through shellfish consumption, it is important to keep in mind the essential functions of many metals in living systems. At least fifty metalloenzymes have been identified (33). These are enzymes which incorporate one or more metal atoms in their normal structure. About twenty incorporate zinc, the best known being carbonic anhydrase and carboxypeptidase. About fifteen copper containing metalloenzymes are known, although their structure is not well understood; ascorbic acid oxidase and various tyrosinases are examples. Some invertebrates have an oxygen carrying protein, hemocyanin, which contains copper, but not any heme groups (33).

The vital role of many trace metals is well established in plant and animal systems. It is even possible that metals such as cadmium, considered non-essential, may prove, in time, to have a biological function. It is also well established that activity of both "essential" and "non-essential" metals, such as mercury and cadmium, depends on their form or state, and whether or not they are bound to a metalloprotein, as described in Section 2.1.3.

2.1.3 Heavy Metal Toxicity. Metals can act as allergens, mutagens, teratogens or carcinogens at the molecular, tissue or organ level (42). They can also be accumulated in humans, and in other organisms, without apparent harm until they are released. For example, lead may be stored in an inert form in bone where it is harmless; but when released, it can be poisonous. Because they are indestructible, metals cannot be metabolically degraded. They can only be eliminated from the body by excretion (42).

In Section 2.1.2 certain metals are identified as being not only useful but essential in living systems. Even these metals can be harmful when present in excess, or when absent, e.g. iron. Metals believed to be non-essential may block activity of essential metals; for example lead can block the utilization of iron in heme synthesis. The behaviour and effects of harmful metals will vary with the organ or tissue in which they are located (42). If attached to a membrane structure they can lead to inhibition of active transport or to permeability of membranes. They can adsorb onto organic molecules such as amino acids and proteins, and form colloids which can enter cells and inhibit enzyme activity. An example is the formation of lead colloids which can inhibit amino levulinic acid dehydratase activity. A mutagenic or carcinogenic potential lies in the inhibition of DNA repair enzymes; and attachment to nucleic acids can disrupt transcriptional or translational processes (43).

The damage done by metals is determined by a number of factors, beginning with the form, or state of the metal. For example, of the

three oxidation states of mercury, each has a quite distinctive effect. Metallic mercury (Hg°) affects the central nervous system; mercurous mercury (Hg_2^{2+}) is slightly irritating, while mercuric mercury (Hg^{2+}) is highly toxic. If ingested it would be corrosive to the gastrointestinal tract, and damage the kidney. Certain metals, such as mercury and lead, can form organometallic compounds by covalently bonding with a carbon atom. Often these compounds, such as methylmercury or tetraethyllead, are the most toxic of all forms (43, 44).

Other factors which contribute to the degree of toxicity of a metal are the dose, the route of absorption, e.g. inhalation or ingestion, the dietary pattern and nutritional state of the person, as well as age and general health. A high dose, for a short term exposure will have a quite different effect from a long term or chronic low dose exposure. Every organ and organ system can be involved in 'metal poisoning', the degree of toxicity being dependent upon the dose. Treatment is possible if the toxic compound has been identified in time. In chronic situations it is often difficult to diagnose correctly.

Another problem is the interaction of more than one metal in the living system. The combined effect of interaction may be (a) synergistic, exemplified by the increased teratogenic effect from a combined injection of cadmium and lead into laboratory animals as compared to the effect from individual injections; or (b) antagonistic, when one factor reduces the effect of another. For example, selenium (as selenite) has been shown to reduce the lethal effect of inorganic mercury in rats (43).

Interactions between metals and macromolecules are probably responsible for the toxic effects of most metals (43, 44). In most cases the receptor for the toxic action is considered to be a macromolecule functioning catalytically, or as a structural or transport component of a membrane. Lipid soluble molecules, such as methylmercury (CH_3Hg) have the ability to diffuse into the membrane where they can block activity better than Hg^0, because, it is thought, the former is more lipid soluble.

It appears that virtually all metals associated with living systems can be harmful under certain circumstances. The Handbook on Toxicology of Metals lists thirty metals (45). Of these, mercury, cadmium and lead are of particular significance because of their known toxicity and their measured increase in the environment as a result of anthropogenic activity. They are therefore presented individually, in brief detail.

Mercury

Mercury, the only liquid metal at 25°C, was a source of mystery, fear and reverence in man's early history. Archeological evidence indicates it was mined in neolithic times. It was evident in a pigment derived from cinnabar in a tomb dating from 2000 BC, on a Mediterranean island. Its role as an environmental pollutant was recorded in the second century, BC, when a mercury mine was closed by Roman senate decree. The mine was causing deforestation and injury to fields and vineyards, as well as polluting streams and killing fish (46).

Mercury is still being mined. Prior to the 16th century its chief use was in medicine and paint. By the turn of this century the chief uses were the manufacture of fulminate and vermillion, the recovery of gold and silver and the making of felt. Now the two principal uses are in electrical apparatuses and in the production of caustic soda and chlorine. Its use in laboratories is extensive (47).

Natural mercury can enter the biosphere as a gas, in lava, in solution or in particulate form. It has been shown to have necrotic effects on plants in the gaseous state (48). In aquatic systems it has been recognized that speciation of trace elements, including mercury, is a primary factor in controlling their behaviour and fate (49).
Mercury can form a wide variety of species in water, which makes it complicated to study. This is due to the comparable stability of its three valence states and to the ability of bivalent mercury to form complexes with many chemical species in solution, including organic ligands

(42, 49). Mercury is more frequently found associated with organic molecules in coastal waters than in the open ocean (49).

Organomercurials represent the most toxic forms of mercury. Their presence in water bodies may be the result of natural processes or of human activities. Methylmercury, one of the most toxic of the organomercurial compounds is known to be formed enzymatically by bacteria and possibly by other systems (50, 51). Non-enzymatic methylation may also be possible (50).

Once in the water or sediments, the mercuric compounds may then be taken up by fish and shellfish, and stored by many of them. Through this process they reached the human population at Minimata, Japan. This is one of the few occasions when a detailed account has been kept of a widespread epidemic of metal poisoning (8) and the only known situation of mercury poisoning in epidemic proportion related to shellfish and fish.

Cadmium

Cadmium occurs in the environment with zinc, and has similar chemical properties. The cadmium/zinc ratio in minerals is 1:100 and in soils 1:1000. It is obtained as a by-product from refining of zinc and other metals. Although cadmium has been recognized as a pollutant for only a short time, it has been part of environmental pollution ever since man started to produce metals from ores which happened to contain it. It is used in a number of industrial processes including electroplating and as colour pigments in plastics and paints. It is also often used in alloys with copper in such things as automobile radiators. Analyses of cadmium in food shows that in "uncontaminated" areas the range is from .001 in potatoes to 1.6 in beef kidney ($mg.kg^{-1}$ wet wt.). In contaminated areas, such as Japan, concentrations of 1 mg $Cd.kg^{-1}$ are found in unpolished rice. The average in uncontaminated areas is .035 $mg.kg^{-1}$ (wet wt.) (52).

Once ingested (or inhaled) cadmium is transported via the blood to other parts of the body and stored mainly in liver and kidneys. The symptoms of acute cadmium poisoning from ingestion are nausea, vomiting,

abdominal cramps and headache. In severe cases diarrhea and shock may develop. The onset of symptoms is usually a matter of minutes upon ingestion of contaminated food or drink (53). Chronic poisoning from ingestion on a large scale has been known to take place only in Japan, from rice contaminated with cadmium.

It has been calculated that a daily cadmium intake via food, by an average adult, would have to be 350 $ug.day^{-1}$ to reach the critical concentration at age 50. The critical concentration is calculated to be 200 $mg.kg^{-1}$ wet wt., in the renal cortex. Chronic cadmium poisoning produces proteinuria and affects the proximal tubules of the kidney, causing kidney stone formation (53).

Cadmium, like other heavy metals is concentrated by shellfish. In a polluted area in Japan, values were reported from 10-110, and 92-420 $ug.g^{1}$ wet weight in shellfish. In the U.S., cadmium concentrations were reported in eastern oysters at 0.1-7.8 $ug.g^{-1}$, and from 0.2-2.1 $ug.g^{-1}$ (wet wt.) in west coast oysters (54).

Lead

Lead is probably the most ubiquitous metal in the environment. Through weathering of mineral deposits of the earth's exploitable lead reserves and through emission from active volcanoes, about 210,000 tonnes of lead are estimated to be released annually into the environment (22). Lead is mined and produced from scrap (secondary lead) which accounts for about 35% of the total world lead supply. It is used to the greatest extent in the storage battery industry (40%), followed by alkyllead production (12%); alloys, pigments and other uses account for the rest. The automobile industry uses over 50% (55).

Lead poisoning (plumbism) has been known for some 2000 years. It is insidious and difficult to diagnose. Chronic lead poisoning in children has been claimed as a major source of brain damage, mental deficiency and serious behavioural problems (22). Lead concentrations in

food products range from undetectable levels to a few $mg.kg^{-1}$ wet weight, depending on the food (16). Fish and seafood were reported as having an average of 0.2-2.5 $mg.kg^{-1}$ wet wt. (55). Much of the anthropogenic lead reaches the marine environment through storm drains, particularly lead compounds associated with gasoline products. To date no case has been reported of lead poisoning in humans arising from contaminated shellfish.

2.1.4 Marine Bivalves as Indicators of Heavy Metal Pollution. The dramatic difference between metal concentrations in marine organisms, the sediments and the overlying water, was pointed out by Klein and Goldberg in 1970 (56). They found mercury concentrations in animals to be 500 times greater than in water and 100 times greater than concentrations in the sediments. (The ratios in ppm dry wt. were 2.1/0.02/0.004, animal/sediment/water). In 1973, Schulz-Baldes reported on the mussel Mytilus edulis as an indicator of trace metals in the German Bight (57). Two years later Goldberg proposed that the ubiquitous mussel be used as the international indicator of heavy metal pollution (58). In 1978 marine scientists from 20 countries met in a workshop to assess the strength and weaknesses of using 'sentinel' organisms to monitor pollution in the seas (4). As biological indicators, mussels and other marine bivalves have the advantages of being sessile and able to concentrate trace metals, as well as some other pollutants. Phillips (2) has published a comprehensive survey, "Quantitative Aquatic Biological Indicators" which explores the various aspects of biological monitoring and gives a list of basic pre-requisites, as follows:

(a) The organism should accumulate the pollutant without being killed by levels encountered in the environment.
(b) The organism should be sedentary in order to be representative of the study area.
(c) The organism should be abundant throughout the study area.

(d) The organism should be sufficiently long-lived to allow the sampling of more than one year-class, if desired.
(e) The organism should be of reasonable size, giving adequate tissue for analysis.
(f) The organism should be easy to sample and hardy enough to survive in the laboratory, allowing defecation before analysis (if desired) and laboratory studies of pollutant uptake.
(g) The organism should tolerate brackish water (59).
(h) All organisms of a given species used in a survey should exhibit the same correlation between their pollutant content and the average pollutant concentrations in the surrounding water, at all locations studied under all conditions (60-62).

Phillips (2) also points out the need to consider the route of metal (and organochlorine) compounds when selecting a "sentinel" organism. While filter feeders are most suitable for uptake of particulate matter from the water column, certain algal species may be preferable for some metals and other contaminants in solution and burrowing organisms may be more suitable to monitor sedimentary pollutants.

Bivalves are being used as pollution indicator organisms in many parts of the world to different degrees. The most extensive "mussel watch" program is in the U.S., as shown in Figure 2 (2). In the California "mussel watch", a number of different studies have been reported as part of the State Water Resources Control Board, water quality monitoring reports (63-65). In one example of this program, trace metal concentrations were measured in the mussel, Mytilus californianus, at 32 stations. Lead, zinc and silver were highest in urban areas. U.S. Food and Drug Administration (FDA) "alert levels" were found at 10 stations, i.e., Cadmium - 1.5, lead - 2.0 and zinc - 30 $ug.g^{-1}$ wet weight. The "alert levels" refer to commercial growing area values, and are not representative of toxic or lethal levels for humans or animals (63).

FIGURE 2 U.S. MUSSEL WATCH STATIONS (2)

In order to cite examples from the multiplicity of studies on heavy metals in marine bivalves, they are separated into two broad and arbitrary categories, with some inevitable overlap. The first category incorporates investigations of bivalves as "sentinel" organisms to monitor heavy metal and other marine pollutants. This category includes those studies of the various factors which affect the bioaccumulation of heavy metals by bivalves. The second category consists of examples of research into the effects of heavy metals on the biology, physiology and biochemistry of marine bivalves.

The first category includes many studies in which metals have been monitored in bivalves in a particular geographical location, e.g. a bay in Australia (61, 66), estuaries in eastern and western Canada (67, 68); England (69); Hong Kong (70); the Mediterranean (71) and Scandinavia (72-74). Some of these, and other studies, look at particular sources or causes of pollution including industrial wastes (61, 75), vessels (76), dredging (77), sewage and wastewater outfalls (78, 79).

Factors which contribute to the uptake of heavy metals by bivalves are: bioavailability of the metal(s); physiological state of the organism and physical or environmental factors such as temperature and salinity, as pointed out by Frazier in a study of the American oyster Crassostrea virginica (80). Many studies have investigated the role of seasonality in bioaccumulation of heavy metals by bivalves. Salinity and temperature, which can affect presence and availability of metals (71, 81) also interact in the preferential uptake of certain metals (82, 83). For example zinc uptake by Mytilus edulis was not affected by changes in temperature and salinity, whereas cadmium uptake was always greater at lower salinities and lead uptake increased at higher salinities. Temperature changes did not significantly alter zinc or lead uptake, but higher temperatures increased cadmium uptake, at lower salinities. The behaviour of copper was erratic under all conditions (82).

Seasonal differences in metal concentrations can be attributed in some cases to the physiological activity of the organism. In a two

year study with hatchery-reared oysters Frazier found that seasonal changes in concentrations of copper, zinc and cadmium in soft tissues were related to gametogenesis and spawning, while manganese and iron were correlated with shell deposition (84, 85).

In recent studies in Vancouver Harbour of trace metal concentrations in Mytilus edulis, Popham and D'Auria found seasonal variations of lead, zinc, copper and iron in mussel tissue, but only in iron and zinc in the surrounding seawater (86, 87). They also found that copper concentrations in the mussels could not be correlated with those in the water.

As briefly discussed in Section 2.1.1, the state of the metals in an aquatic environment determines their availability to the biota. For example, Grieve and Fletcher studied the interactions between zinc and suspended sediments in the Fraser River Estuary. They found that adsorption, desorption and circulation keep the heavy metals in coastal zone sediments and waters (88). Lu and Chen report that different metals migrate between surface sediments and the seawater, depending on redox conditions. They found three general trends: reducing conditions released more iron; oxidizing conditions released more cadmium, copper, nickel, lead and zinc; mercury and chromium showed no significant change (89).

Several field and laboratory studies have investigated the factors controlling the availability of sediment-bound metals to marine organisms. In a study of a deposit-feeding bivalve, Scrobicularia plana, in twenty estuaries in England and France, it was found that biologically available lead to this organism was controlled by the concentration of iron. The ratio, Pb/Fe in 1N HCl extract of surface sediments allowed the authors to predict the amount of lead available to S. plana. It was not determined if hydrous oxides of iron could bind lead in the sediment, making it unavailable, or if the two metals competed for uptake by the bivalve (90). In either case, Phillips (2) points out that measurements

of lead concentrations in S. plana would give erroneous information as to lead contamination in the surrounding sediments.

Genest and Hatch (91) measured cadmium, copper, iron and lead in sediments and the tissues of Mercenaria mercenaria and found the tissue concentrations varied significantly with the season. In the sediments cadmium did not vary with season or site, whereas copper, iron and lead were site dependent (91). The availability of copper to marine bivalves (and a shrimp) was found in a laboratory study to be related to cupric ion activity (92). More than 50% of the cupric ion (Cu^{+2}) was bound to organic parts of the sediment and therefore unavailable to suspension feeders. However a deposit feeder, Macoma inquinata doubled its copper content in two months. The authors suggest that it is better to measure ionic and weakly complexed copper by anodic stripping voltammetry than trying to measure total copper, to learn the amount that is bioavailable (92).

Two laboratory studies endeavoured to learn how concentrations of metals in the sediments and the type of sediment affected bioavailability of certain metals. In the first experiment it was found that the test animals accumulated more metals from the sediment with the lesser concentrations of copper, zinc, cadmium and lead, indicating metal interaction within the sediment (93). In the second study, the edible cockle (Cerastoderma edule) was tested with four sediments containing cadmium. From one of these, biogenic calcium carbonate, the cadmium was readily available and less so from precipitated $CaCO_3$. It was unavailable from sediments of iron and manganese oxide. The authors conclude that availability in this case depends on the ability of the cadmium to desorb (94).

The final types of studies included in the first category are those which consider the statistical problems inherent in monitoring programs. Gordon et al. measured concentrations of eight metals in two populations of Mytilus californianus from two areas. They found that 'within' populations variability was such that they needed 10 to 20

individual organisms to give maximum resolution and maintain cost effectiveness (95). Similar results were found with three different populations of oysters. It was found that a different minimum sample size was required for each population (96). A recommended sample size is twenty- five individuals for biochemical measurement (4). Popham and D'Auria have proposed statistical models for the estimation of metal concentrations in seawater from those measured in mussels (97). Statistical considerations are discussed further in Methods (Appendix VII).

Early work, on the second category of studies, i.e. effects of heavy metal population on the biology and physiology of marine organisms, including bivalves, was reviewed by Waldichuk (98). The toxicity of various metals has been determined as LC_{50} values to adult (99-101) and larval bivalves (102-104). (LC_{50} = lethal concentration, fatal to 50% of the sample). Representative LC_{50} values for selected metals on some bivalve eggs and larvae are given in Table 4 (101). In one study (102) mercury and silver were shown to be the most toxic of several metals to oyster larvae, followed by copper and zinc. Cadmium, lead and nickel were appreciably less toxic and arsenic, manganese and aluminum were relatively non-toxic. A later study showed that even lower concentrations of some metals were toxic to mussel and oyster embryos (103). Heavy metal (cadmium) taken up by adult female oysters did not appear to affect their fecundity, but did have a deleterious effect on larval viability (104). The effects of heavy metal stress, other than toxicity, were the subject of several investigations, which looked at the response of embryos and survival and growth of larvae under metal stress (105, 106) and synergistic effects of temperature and salinity with individual metals (107, 108). Metal effects were found to be minimal when salinity and temperature were optimal.

A number of studies have concentrated on the ability of adult bivalves to depurate heavy metals (109-116). In one study (109) oysters accumulated mercury (as acetate) at concentrations of 10 and 100 ppb for

TABLE 4: ACUTE TOXICITY OF HEAVY METALS TO MARINE BIVALVES (101)

ORGANISM			METAL	EXPOSURE PERIOD	LC_{50} (ppb)
Crassostrea virginica			Cadmium	48 hrs	3,800
"	"	eggs	Silver	48 hrs	5.8
"	"	larvae	"	12 days	25.0
"	"	eggs	Mercury	48 hrs	5.6
"	"	larvae	"	12 days	12.0
Mercenaria mercenaria					
"	"	eggs	Silver	48 hrs	21.0
"	"	larvae	"	10 days	34.4
"	"	eggs	Mercury	48 hrs	4.8
"	"	larvae	"	10 days	14.7

sixty days. While there was some decline after 18 days, e.g. from 18,000 to 15,000 ppb in the 10 ppb group, and 115,000 to 65,000 ppb in the 100 ppb group, the animals were not cleaned out in six months. Factors found to affect accumulation were size, spawning and temperature. Smaller animals (less than 7 gm) accumulated more metal per body weight (109-111). In these and other studies (e.g. 117) highest metal concentrations were found in the gills. After the first three days the order of concentration was gills, digestive tract, mantle, gonads, muscle. Later the digestive tract showed higher concentrations than gills. Depuration was found to occur first from the gills and digestive tract, then from the mantle, gonads and muscle. The importance of size and reproductive condition to accumulation and depuration were noted in two reports (113, 116). Latouche and Mix (113) noted that larger mussels had more nickel, copper and cadmium in somatic tissue than in gonadal tissue whereas smaller (assumed younger) animals had more manganese. They also noted an increase in copper and nickel in somatic tissue which may be a stress response "manifested in appearance of new byssal threads". Females of Mytilus californianus were reported to contain more copper and zinc than the males in gonadal tissue whereas lead was the opposite (118, 119).

Information pertaining to direct physiological and biochemical responses by adult bivalves to heavy metal stress does not abound. Early studies reported by Calabrese et al. (101) showed increased respiratory rates to elevated concentrations of silver in oysters, mussels and clams, in laboratory experiments. While changes in concentrations or synthesis rates of carbohydrates, lipids and proteins can indicate general stress, it is difficult to relate such changes to pollutant stress. The significance of the occurrance of metallothionein-like proteins in relation to metal stress is controversial. Several studies have shown the induction of such metal-binding proteins in clams and mussels (37, 38-41). Brown and Chatel (120) found that synthesis of metal-binding proteins was less efficient in response to acute exposure than to chronic exposure. In the former case metals were bound 1.6 times over control values.

Changes in concentration or activity of metallo-enzymes and lysosomal enzymes have also been proposed as a means of elucidating metal stress (4). Changes in free amino acids accompanied by byssal thread production have also been used as a biochemical indicator of heavy metal stress (121). George et al investigated the metabolism of zinc and other metals in a combined ultrastructure and biochemical study of mussels (122, 123). Viarengo et al (124) compared several biochemical parameters in mussels gathered from polluted and unpolluted areas. They found that polluted mussels had significantly decreased rates of protein and RNA synthesis, and amino acid uptake. They also had increased levels of a low molecular weight, thionein copper-binding protein.

Behaviour is another factor used in the monitoring of metal stress in bivalves. Although there are not as many overt manifestations in sessile as in mobile animals, some have proven useful. Byssal thread formation has been considered (121) and the burrowing activity of clams. McGreer (125, 126) has shown effects of metal contamination in sediment by various parameters including behaviour of Macoma. Eisler (127) strongly recommends the use of behaviour responses as a means of identifying and measuring marine pollutants.

A tried-and-true method of determining the general health of bivalves is the use of nutrition or condition index. This is the ratio of dry flesh weight to total volume (with shell) or to total weight (flesh + shell). Although such a method dosen't indicate a specific cause of stress or deterioration, it is a comparatively simple way to keep a weather eye on the general state of a bivalve community. This and a number of more complex methods of determining biological fitness of bivalves for monitoring purposes are discussed in the International Mussel Watch (4).

2.2 Polycyclic Aromatic Hydrocarbons (PAH)

Polycyclic aromatic hydrocarbons (PAH) [also called polynuclear aromatic hydrocarbons (PNA)], represent a major class of carcinogens,

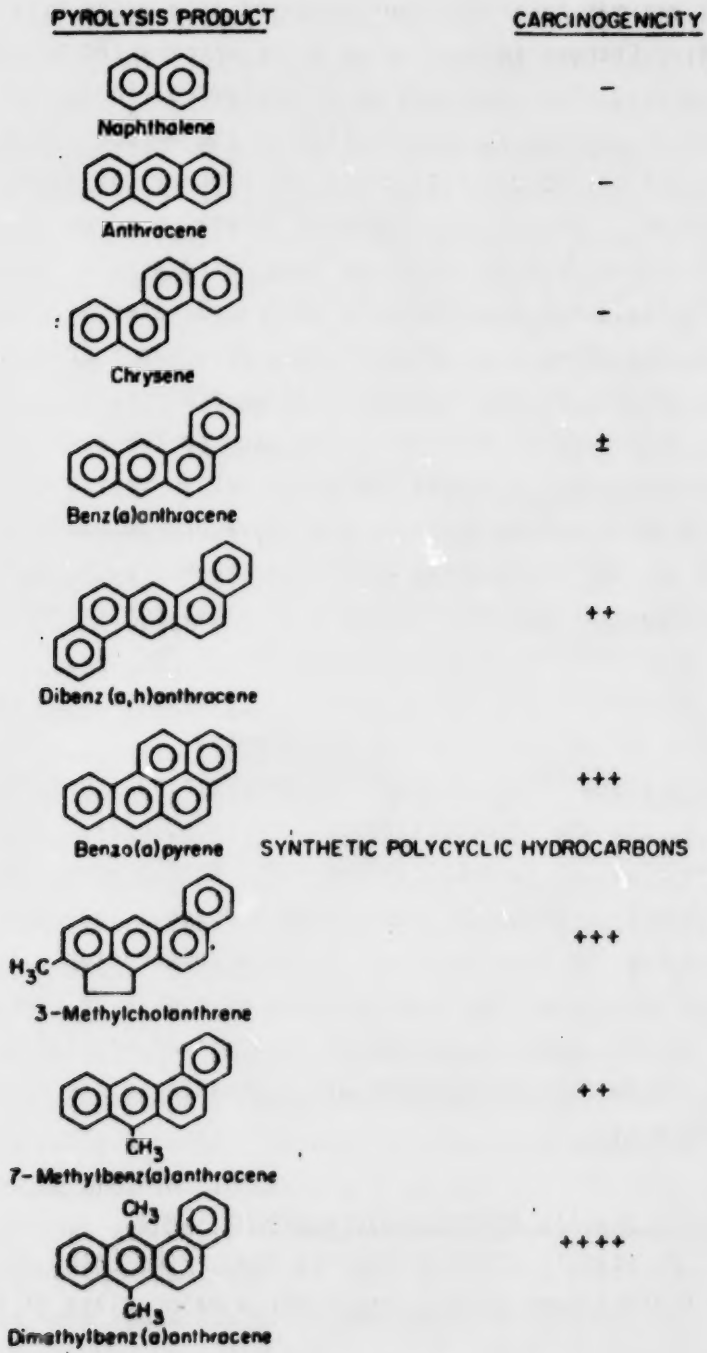

FIGURE 3 NATURALLY OCCURING AND SYNTHETIC POLYCYCLIC AROMATIC HYDROCARBONS

consisting of multiple fused benzene rings (Figure 3). They are found in air, soil, water and bottom sediments throughout the world, especially in industrialized areas. They are products of alkene free-radical conjugation during incomplete combustion of fossil fuels, and are therefore major exhaust components from transportation, industrial energy sources, refuse burning, and cigarette smoke (128). They also may be produced by natural metabolism (129). Benzo(a)pyrene (B(a)P) is a major carcinogen among the PAH, according to the classification of Lo and Sandi (130). It is the most important and often the only PAH measured in food (130)(See Section 2.2.2).

2.2.1 PAH in the Marine and Estuarine Environments. PAH in the marine environment have been associated frequently with oil spills, which are not the subject of this report. However, PAH may enter coastal waters from other diverse sources, including municipal wastewaters, as motor exhaust (128, 131) or as decaying animal or vegetable matter (129). PAH also find their way into the oceans by seepage from geological formations, runoff from land, blowouts from off-shore oil wells and shipping practices of bilge and tank washings (132). Plant biosynthesis of PAH has also been reported (133, 134) but it is more likely that PAH resulted from contamination in the laboratory (135, 136).

Two general processes are considered to determine the fate of PAH in natural water, namely transport and transformation. Transport alters only the physico-chemical form abiotically, and results in dispersion, sorption or volatilization; or PAH are biotically accumulated. Transformation may be brought about abiotically by oxidation or photolysis, or biotically by microbial degradation or metabolism (137).

In order to get a world-wide perspective on PAH levels in marine sediments, as distinct from total hydrocarbons, B(a)P was analyzed in sediment samples from many wide-spread locations, including harbour and non-harbour sites on the Pacific coast of North America, the

MacKenzie River Delta and Beaufort Sea, the northwest coast of Alaska and a German oil port (135). The median level in the Pacific coast harbours was 105 $ug.kg^{-1}$. In non-harbour sites the value was 0.4 $ug.kg^{-1}$, and in most uninhabitated areas it was below detection. The highest B(a)P value was 1500 $ug.kg^{-1}$ in sediments from Wilhelmshaven oil port (135).

2.2.2 PAH in Food. Besides reaching man through the routes mentioned above, PAH can reach man through drinking water and food (138).

Raw meat and fish do not usually contain PAH, but they are commonly found in meat or fish that is smoked and cooked in various ways (138, 139). The amount of PAH is related to the kind and degree of smoking or cooking. B(a)P values found in some of the above foods are shown in Table 5.

A survey of commercial shellfish (139) showed that PAH contamination occurs in organisms from "unpolluted" waters, as well as polluted waters. A substantial number of shellfish samples contained more than 5 $ug.kg^{-1}$ B(a)P, with two samples containing more than 25 $ug.kg^{-1}$ (wet wt.) (139). This same survey showed that most commercial seafood samples contain at least a trace of B(a)P. Data from the survey are given in Table 6.

2.2.3 PAH Toxicity and carcinogenicity. There is little information on the toxicity of PAH administered orally to experimental animals. The systemic acute dose LD_{50} is usually high compared to the dose necessary to produce carcinogenic effects. However, PAH induced cancers in laboratory animals are well documented (138). B(a)P specifically has produced tumors in rats, hamsters, guinea pigs, monkeys and other mammals when administered by oral, skin and intratracheal routes (138).

2.2.4 Marine Bivalves as Indicators of PAH Pollution. Clams, oysters and mussels readily accumulate PAH from contaminated waters and

TABLE 5: BENZO(a)PYRENE CONCENTRATIONS IN SELECTED FOODS (138)

FOOD TYPE	B(a)P CONCENTRATION (ppb)
Charcoal broiled steaks	5.8
Smoked ham	3.2
Barbecued ribs	10.5
Heavily smoked bacon	3.6
Apple	0.02 - 8.3
Banana	0.02
Pear	1.9
Plum	0.04 - 29.7
Wheat sprouts	60.0
Rye seedling	10.0 - 20.0
Parsley leaf and stem	24.3
Mushroom	7.0
Lettuce	8.6
Radish roots	1.2
Tomatoes	0.2
Cabbage	12.3 - 20.9
Potatoes	0.2

TABLE 6: BENZO(a)PYRENE IN COMMERCIAL SEAFOODS (139)

SPECIES	ORIGIN	TYPE[a]	B(a)P ng/g wet weight[b]
Molluscs			
Abalone	Canada	f	0.6
Clams	Canada	f	ND, 0.6, 0.6
	Italy	t	8.1
	Japan	t	ND, 5.6, 5.8, 6.3
	Korea	t	0.8
	Malaysia	t(v)	1.8
Cockels	England[c]	t	18, 19, 36
Cuttlefish	Spain	t(s)	0.2
Mussels	Canada	t	ND
	Denmark	t	3.3, 5.1
	England[c]	t	5.2, 5.3, 5.7, 25
	Germany	t(s)	1.6
	Spain	t(s)	2.0
Oysters	Canada	f	0.4, 1.6, 2.0, 3.2, 6.2
	Japan	t	0.7
Scallops	Canada	f	0.3
Squid	USA	t	ND, ND
	Spain	t(s)	1.0
Crustaceans			
Crab	Canada	f	ND, ND, ND, 0.2, 0.2, 0.3
	USA	t(s)	2.1
Lobster #1	Canada	f	claw: 3.8[d]
			tail: 7.9[d]
			legs: 5.1
Lobster #2	Canada	f	claw: 0.8[d]
			tail: 2.5[d]
Lobster #3	Canada	f	claw: 2.0[d]
			tail: 2.6[d]
Prawns	Canada	f	ND
	USA	f	ND
Shrimp	Canada	f	ND
	USA	t	ND
	Greenland	t	0.3

TABLE 6: BENZO(a)PYRENE IN COMMERCIAL SEAFOODS
(Continued)

SPECIES	ORIGIN	TYPE[a]	B(a)P ng/g wet weight[b]
Vertebrate fish			
Arctic char	Canada	t	ND
Cod	Spain	t(s)	1.5
Cod liver	Germany	t	ND, ND
Dover sole	Canada	f	ND
Mackerel	Spain	t(s)	0.7
	Yugoslavia	t(v)	2.6
Salmon	Canada	f	ND
	Canada	t	ND
Sardines	Morocco	t(o)	0.2
	Norway	t(o)	ND
	Spain	t(s)	ND
Trout	Portugal	t(o)	1.4
Tuna	Japan	t	ND

[a]f = fresh or fresh-frozen, t = tinned or bottled, with soya (s), olive (o), or unspecified vegetable (v) oil.
[b]Multiple values for a species and country are for samples from different suppliers, unless otherwise noted. ND = less than 0.1 ng/g.
[c]Individual tins from the same supplier.
[d]Average of two determinations.

sediments. Uptake mechanisms are not well understood, but two distinct processes are suggested: (1) PAH, attached to organic or inorganic particles are transferred whole to the bivalve; (2) PAH is partitioned between sedimentary particles and the water, before reaching the organism. In the latter case the organism receives the more water soluble complexes and concentrations would not directly reflect sedimentary levels (10).

Although PAH contamination in shellfish (oysters) was first reported in 1957 (140) and even earlier in barnacles (141), few investigations were published until the early 1970s. Many of the later studies were related to oil spills and contamination of shellfish and other organisms by petroleum hydrocarbons in general. Early European reports of substantial PAH contamination in organisms from presumably uncontaminated areas was reviewed by Zobell (134). DiSalvo, et al (10) proposed that tissue hydrocarbon burden in mussels be used to monitor environmental hydrocarbon insult. Dunn and Stich (131) suggested that B(a)P in mussels be measured for this purpose, and subsequently published a procedure to monitor chemical carcinogens in coastal waters (142). They suggested that B(a)P in mussels could be a general indicator of other related carcinogens in much the same way that Escherichia coli is used to indicate coliform contamination.

A number of studies have investigated uptake and release of hydrocarbons, including PAH, by bivalves (143-147). From several studies it appears that mussels and other bivalves cannot metabolize B(a)P (148). It has also been suggested that bivalves sequester some components in compartmentalized tissues that have limited accessability for exchange when placed in a clean environment (10). Another point is that uptake and release rates are related to the quantity of pollutant and time during which it is presented to the organisms. High concentrations, given for short periods, are released by the organisms at a more rapid rate than lower doses administered over a long period.

An experiment to determine the depuration rate of environmentally contaminated mussels (45 ug.kg^{-1} wet wt.) resulted in a half-life of over two weeks for B(a)P (143). This is in sharp contrast to half-life of 1.3 days in mussels, dosed with 1,400 ug.kg^{-1} naphthalene for four hours; or 3.5 days for mussels contaminated with 250,000 ug.kg^{-1} aliphatic hydrocarbons (wet weight) (145). The half-life period of 2 to 3 weeks for B(a)P in the environment does compare with a half-life period of 4 to 5 weeks, for total environmental aromatic hydrocarbon content of mussels in nature (143). PAH in general have been found to have a slow release rate from bivalve molluscs (146).

Many of the early studies on effects of petroleum hydrocarbons, which include PAH, were done simply with different types of oil. Extended exposure of mussels to diesel fuel of medium concentration resulted in 70% mortality compared to controls, and in general debility of the mussels, measured by 'condition factor' (145). Other studies showed morphological and physiological effects on bivalves contaminated with outboard motor effluent (149) and waste motor oil (150). Water soluble extracts from crude oil were highly toxic to the gametes and embryos of oysters, and affected shell development and swimming of the larvae (151). Effects of B(a)P specifically on these stages are not known. A study of cellular response to PAH in mussels showed increased activity of microsomal enzyme systems, related to mixed function oxygenases in blood cells. There was also lysosomal destabilization as has been reported in mammals (152).

While some studies are directed towards a general assessment of hydrocarbon pollution of coastal area (e.g. 153, 154), others are concerned with effects on important commercial shellfish industries (146). Still others have concentrated on carcinogenic contamination of shellfish as food for human consumption (139).

Petroleum hydrocarbon pollution was measured in an Australian Bay by transferring uncontaminated mussels from a clean area to 20 stations in other areas of Westernport Bay for monitoring purposes (4).

Highest contamination values (600 to 1,200 parts per million (ppm) dry weight) were found in mussels from an area near a refinery outfall. The amount of hydrocarbons diminished in samples at increasing distances from various input areas. A similar program was carried out in Port Phillips Bay at 56 stations. Petroleum hydrocarbon contamination was assumed to have come from mixed sources, e.g. ships, industrial discharges and storm drains. In both bays there was a difference of at least two orders of magnitude in hydrocarbon contamination between mussels sited close to known or suspected sources and those some distance away (4).

Monitoring of PAH or other petroleum hydrocarbons in the U.S. and Canada also indicat[e] more contamination in harbour areas, with indications that in so[me] cases the source of contamination was fuel or crude oil. In other cases pyrolytic sources were responsible for aromatic hydrocarbons (4). In a study along the Atlantic seaboard in Canada several PAH were monitored in bivalves at 70 stations. Higher concentrations were found in animals close to creosoted wood structures (4). Dunn and Stitch (131) also found creosoted wood was a prime factor in PAH pollution, as discussed in more detail in Section 3.2.

2.3 Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls have been manufactured and used since 1930 in numerous products including plasticizers, lubricants, hydraulic and insulating fluids, heat and transfer agents and others. Most applications have resulted directly from PCBs being relatively nonvolatile, insoluble in water, soluble in organic compounds (especially hydrocarbons), and having high dielectric constants. They are relatively inert towards acids, alkalies and other corrosive chemicals. PCBs are stable towards oxidation and resist combustion at temperatures above their boiling points. Temperatures in excess of 1000C are required for their complete combustion (155). The same properties which make PCBs valuable in their various uses are detrimental in the environment, where PCBs are accumulated and retained in living systems.

PCBs were first recognized as an environmental contaminant in 1966 when they were discovered by Jensen (156) as an unknown compound in a sample of pesticide residues. Shortly after they were reported from many parts of the world (157, 158). Studies in Canada, summarized in a report by the Canadian Task Force on PCBs (159) have demonstrated that PCBs are ubiquitous contaminants of biota associated with inland and marine aquatic ecosystems. "PCBs actually constitute a major portion of the chlorinated hydrocarbon residues found in fish and birds of the North Atlantic, the Gulf of St. Lawrence, and Great Lakes (160)". A report on PCBs in British Columbia to 1976 points out evidence of PCBs in sediments and biota around industrial areas of Vancouver (161).

2.3.1 PCBs in Marine and Estuarine Environments. PCBs in marine waters near industrial areas vary from 13 $ng.l^{-1}$ in the northwest Mediterranean to 320 $ng.l^{-1}$ in the surface waters of Tokyo Bay. Concentrations were much lower in nearby oceanic surface waters (3.0 $ng.l^{-1}$) and decreased with depth (162). PCB effects on marine organisms, including oysters, are listed in Table 7. The data indicate that direct mortality following exposure to PCBs is probably less critical than sublethal effects such as hepatic enzyme induction, impairment of reproduction and reduction of species diversities in invertebrate communities (160).

PCBs in the aquatic environment are frequently measured with DDT residues or other pesticides. Three years later, after their discovery, Jensen reported contamination of a wide variety of marine organisms including many species of fish and birds (11). In California highest concentrations of PCB were found in mussels near sites of greatest vessel activity. It was suggested that contamination may have come from antifouling paints manufactured before the development of any restrictions on PCB content (163). Another common source of PCBs was outfall systems in major urban areas, as a study of PCB contamination in the Southern California Bight demonstrated (164). The fact that PCBs have found their

TABLE 7: EFECTS OF PCBs ON AQUATIC ORGANISMS[a] (162)

CONCENTRATION IN WATER (ug/l)	ANIMAL	EFFECT	
		DESCRIPTION	SEVERITY[b]
10 - 100	Oyster	Decreased shell growth	C
	Shrimp (Penaeus sp.)	Mortality	C
	Scud (Gammarus sp.)	"	B
	Crayfish	"	B
	Channel catfish	"	B
	Bluegill	"	B
	Pinfish	Degeneration of liver tissue	B-C
1.0 - 10	Shrimp (4 species)	Mortality	B-C
	Rainbow trout	"	B
	Pinfish	"	B
	Spot (fish)	"	A-B
	Oyster	Decreased shell growth	A-B
	Blue crab	Mortality	A
	Spot (fish)	Degeneration of liver tissue	B-C
	Fathead minnows	Mortality and reduced growth	B
	Waterfleas (Daphnia sp.)	Mortality and decreased fecundity	B
	Flag fish	Mortality and reduced growth	C
0.1 - 1.0	Ciliates	Significant reduction in population growth rate	C
	Shrimp	Mortality	A-B
	Marine invertebrate communities	Reduced species diversity	A-B
	Shrimp (Penaeus sp.)	Increased prevalence of viral (Baculovirus) infection	B-C
	Midge larvae (Tanytarsus sp.)	Mortality	B
	Diatom (Rhizosalenia sp.)	Reduced population growth rate	A
	Mixed marine phytoplankton cultures	Reduced species diversity	A-B
	Sheepshead minnow (embryos and fry)	Mortality	B-C

[a]Based on the toxicological data available for Aroclors 1248, 1254, and 1260.
[b]Severity in terms of estimated deviation from control value: A = 0-33%; B = 33-66%; C = 66-100%.

way into ecosystems around the globe was well documented by Risebrough et al (157). Because PCBs are not water soluble they become concentrated in the sediments and are found in greater quantity in benthic organisms close to the bottom than in organisms higher in the water column.

2.3.2 Marine Bivalves as Indicators of PCB Pollution. Reports of marine organisms concentrating PCBs at values high above ambient levels have included bivalves. Oysters were cited as concentrating PCBs to a factor of 85,000 in one study and 100,000 in another (165). The latter study reported even higher factors for shrimp (230,000) and fish (670,000).

A study in the Medway Estuary measured PCB concentrations in mussels (Mytilus edulis) and other organisms. The mussels at all locations concentrated PCBs to a greater degree than did crabs or periwinkles or worms. PCB levels in the mussels ranged from 70.4 to 162.0 $ng.g^{-1}$ (wet wt.) The concentrations decreased downstream, away from industrialized areas (166). A number of animals in the English Channel and North Sea were analyzed for PCBs, DDT and DDT breakdown products, in different tissues (12). Concentrations were greater in the digestive gland of the one bivalve (scallop), with the value of 0.24 $ug.g^{-1}$ wet wt. as compared to 0.048 $ug.g^{-1}$ in the adductor muscle. When analyzed per lipid weight, the values are much greater in the adductor muscle, the highest value being 29 $ug.g^{-1}$ lipid, compared with 2.7 $ug.g^{-1}$ in lipid of the digestive gland (12).

A survey of Puget Sound, Washington reaffirmed that the major input of PCBs was from municipal and industrial outfalls, with accumulation greatest near the source, because of the low water solubility of the compounds. The PCB levels in mussels ranged from 10 to 210 $ng.g^{-1}$ wet wt., with a mean of 44 $ng.g^{-1}$ (167).

2.4 Summary

The foregoing brief account of toxic contaminants in the sea illustrates the problems and dangers inherent in disposing of toxic

chemical wastes in the nearshore marine environment. The problems of monitoring marine pollution are as great as controlling it. In summary,

1) To date heavy metals make up the largest bulk of known toxic contaminants in the marine environment, followed by an ever increasing number of identified organochemicals.

2) Of the metals, mercury, cadmium and lead are considered the most pernicious and dangerous to human health. Lead and mercury from natural sources contribute a large part of the marine waters load, although quantities from anthropogenic sources continually increase, particularly lead.

3) While the above three metals are given special attention, others, such as copper, zinc, silver and nickel are also found in increasing quantities in coastal waters.

4) The acute toxicity of metals (LC_{50} or LD_{50}) has been established for many organisms particularly fish which have been the preferred test organism for some time.

5) Adult marine bivalves accumulate heavy metals and hence are not suitable for toxicity tests, but have proven valuable as indicator organisms. Embryonic and larval forms of invertebrates, however, are particularly susceptible to metal toxicity.

6) Although adult invertebrates accumulate heavy metals, apparently with little damage, at concentrations much greater than the surrounding environmental levels, chronic effects have been shown to occur.

7) Efforts are being made at the international level to set up programmes in which mussels and other suitable bivalves are used to

monitor marine pollution, with cautionary advice as to the organisms to be used in different waters, and the methods applied, so that results are comparable from place to place and through time. Consideration of the behaviour of specific contaminants in the marine environment is also necessary in determining the proper "sentinel" organism.

8) Bivalves also accumulate organochemicals. Inherent problems lie in separating and identifying certain compounds, e.g. PCB and DDT were at one time measured together.

9) Carcinogenic compounds, such as PAH are becoming more predominant in the marine environment from oil spills, marine vessel activity, and in the nearshore environment from storm drains which carry products of automobile fuel combustion into the sea. It has been proposed that Benzo(a)pyrene, one of the most studied carcinogenic PAH, be used as an indicator of general PAH pollution.

10 Mussels and other bivalves have been shown to accumulate B(a)P in a manner similar to PCBs.

3 CHEMICAL POLLUTANTS IN B.C. BIVALVES

The question of pollution in Georgia Strait was discussed recently by Waldichuk at the Symposium on Fisheries and Oceanography of the Strait of Georgia (15) and earlier was the subject of a study by Parsons (14). Metal pollution, specifically mercury, cadmium and lead in the B.C. environment has been reviewed in Department of the Environment reports and include references to marine pollution (18-20). PCB pollution in B.C. was the subject of a recent report by Garrett (168). It summarizes data from 1976-1981 and includes information about PCBs in aquatic invertebrates.

Lists of toxic chemicals in the environment are being developed under a variety of legislative mandates, including the Environmental Contaminants Act. The legislation relates to the manufacture, use, disposal, and levels in food and water. To date environmental alert or "danger" levels have not been established for any chemicals in marine waters (169).

In the discussion that follows, contaminants levels in bivalves are reviewed in three ways: (1) as results from specific monitoring programs in various industry sectors, eg. mining; (2) as results from surveys for specific contaminants, eg. PAH and; (3) as results from general environmental quality monitoring programs, e.g. Fraser River. Although there is some overlap between sections (e.g. trace metal levels are discussed in Sections 3.1, 3.4 and 3.5) it was felt that this manner of data presentation would enable the reader to relate specific contaminant levels to specific sources.

Data for all contaminants reviewed in this report is presented in Appendices II-VI.

3.1 Heavy Metals - Sources

A number of individual studies by government, university and consulting scientists have investigated specific pollutants or sources of pollutants in B.C. coastal waters. None has been directed to toxic

chemical pollutants in relation to commercial bivalves, but most have included bivalves in their sampling or as test organisms. These studies pertain to pollution from sewage outfalls, storm drains, dredging, pulp mills, mines, a chlor-alkali plant and harbour activities. Specific trace metal studies are outlined individually in sections 3.1.1 to 3.4.2. Studies pertaining to municipal outfalls and the Fraser Estuary are presented in Sections 3.4 and 3.5.

A general survey of trace metals in marine organisms was instituted in 1971 by the Habitat Protection Division and Environmental Protection Service of Environment Canada (170). A broad assembly of animals was collected for metal analysis from the coastal waters of southern B.C. at 35 stations close to urban and industrial areas, as shown in Figure 4. Collections were made between 1971 and 1973 (Appendix II, Table 1), and again in 1976-1977. The data were obtained to provide a baseline for monitoring of contaminants in B.C. coastal waters, close to highly populated areas.

Molluscan samples included oysters (Crassostrea gigas), mussels (Mytilus edulis) and clams (Protothaca staminea, Saxidomus giganteus, Tresus capex) and unidentified aggregates. Oysters were collected from eleven sites, mussels from five and clams from three. During the 1976-77 period, only oysters were analyzed for metals.

The concentrations of mercury, cadmium, zinc and copper found in oysters in 1976-77 are given in Appendix II, Table 2. No areas of elevated values, indicating "hot spots" were found in this survey of bivalves. A few high values of mercury were found in dogfish, Squalus acanthas (2.03 $ug.g^{-1}$ wet weight) and the crab, Cancer productus (0.65 $ug.g^{-1}$, wet wt.).

Subsequent investigations of heavy metal pollution in B.C. waters have been directed toward specific sources and in some cases specific metals. Following are resumes of these studies. In all cases shellfish have been included as test organisms, and in some cases they are the only samples analyzed. Studies on metal pollution in the marine environment which do not involve bivalves are not included.

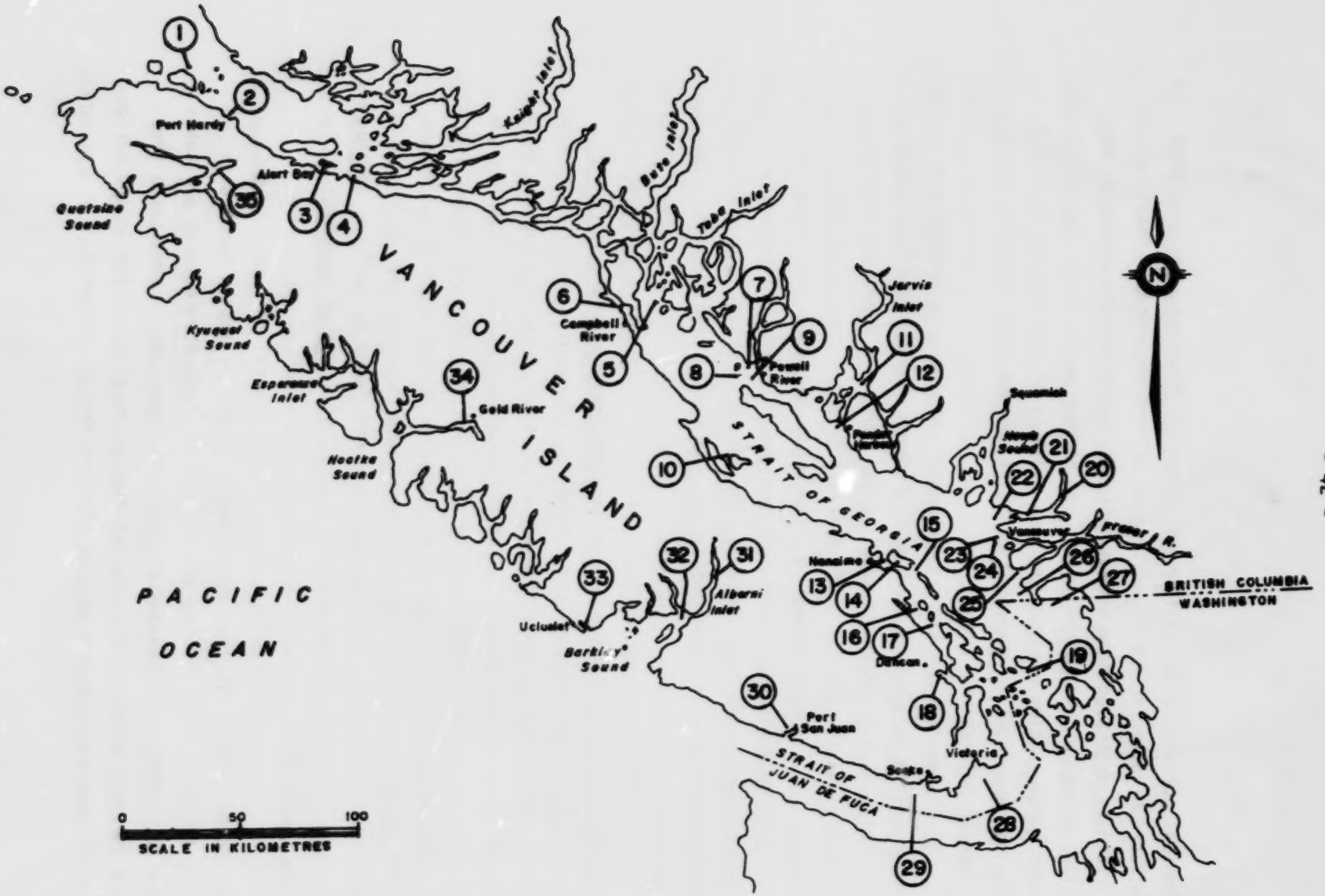

FIGURE 4 SAMPLING SITES IN COASTAL WATERS OF SOUTHERN BRITISH COLUMBIA TRACE METAL SURVEY - 1971 - 1973 (170)

3.1.1 Mercury Contamination From a Chloralkali Plant

The principal industrial use of mercury is in the chloralkali process in which a flowing cathode of metallic mercury is used in the simultaneous production of chlorine and caustic soda, by electrolysis of brine solution (47). Of the three chloralkali plants which discharge wastes into Georgia Strait and environs, the FMC Chemical Plant at the head of Howe Sound is the only one which uses the mercury cell process (15). Investigations in 1969-70 of possible mercury contamination of the Sound resulted, on April 2, 1970, in "an immediate closure of upper Howe Sound to both commercial and sport fishing pending the determination of mercury content of aquatic organisms" (15). Efforts by the FMC plant to remove mercury from the effluent resulted in a decrease from 9,000 gm to 100 gm mercury daily discharges (15). The ban on salmon was lifted the same year, (June, 1970). The ban remained on shellfish and groundfish until May, 1978, because of the ability of these organisms to accumulate the metal and sequester it.

Bivalves and other organisms were sampled to measure mercury contamination over the years in Howe Sound, at stations depicted in Figures 5 and 6. The amount of mercury in crabs decreased significantly, from 13.4 $ug.g^{-1}$ to a mean level of 0.15 $ug.g^{-1}$. Not as many molluscs were sampled, but a difference was seen in mussels (Mytilus edilus) which ranged from 0.02 to 0.07 $ug.g^{-1}$, and in the oyster (Crassostrea gigas) with a range of 0.05 to 0.16 $ug.g^{-1}$ (171). Data are given for mercury in mussels from 1972-75, in Table 3 of Appendix II. In the early studies, Zone 1, nearest the chloralkali plant (Figure 5) had statistically higher concentrations of mercury, though still below 0.5 $ug.g^{-1}$ (wet weight). The other zones ranged between 0.03 $ug.g^{-1}$ and 0.13 $ug.g^{-1}$.

The most recent data (Appendix II, Table 4) collected by EPS in 1981 shows lower concentration at the numbered sampling stations (Figure 6). No collections were made in zones 1,2 and 3. Bivalves samples at stations 7 and 8, closest to the head of Howe Sound, had similar mercury levels to those collected from the mouth of the Sound. The high levels of

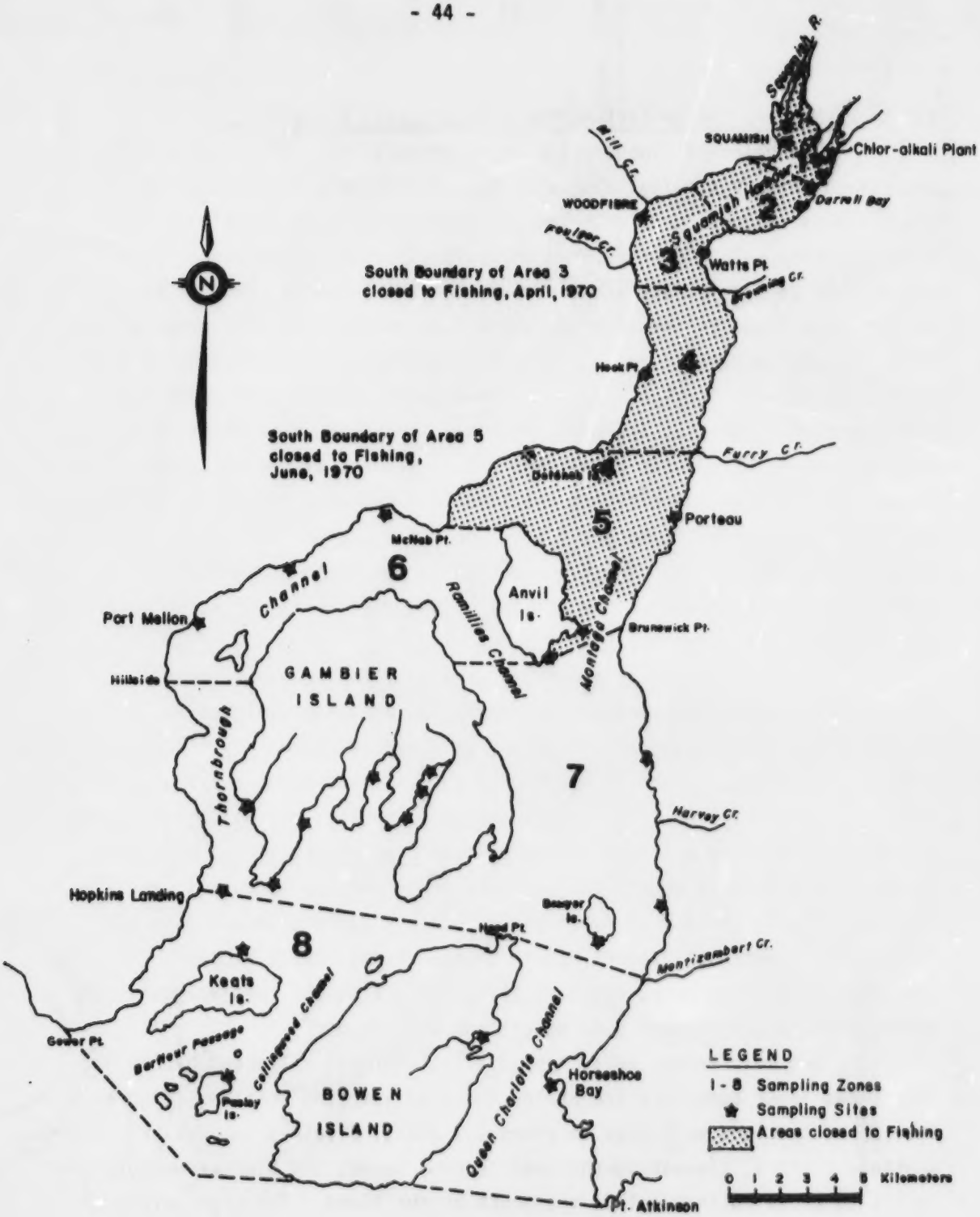

FIGURE 5 TRACE METAL SAMPLING ZONES IN HOWE SOUND (1972 - 1975) (171)

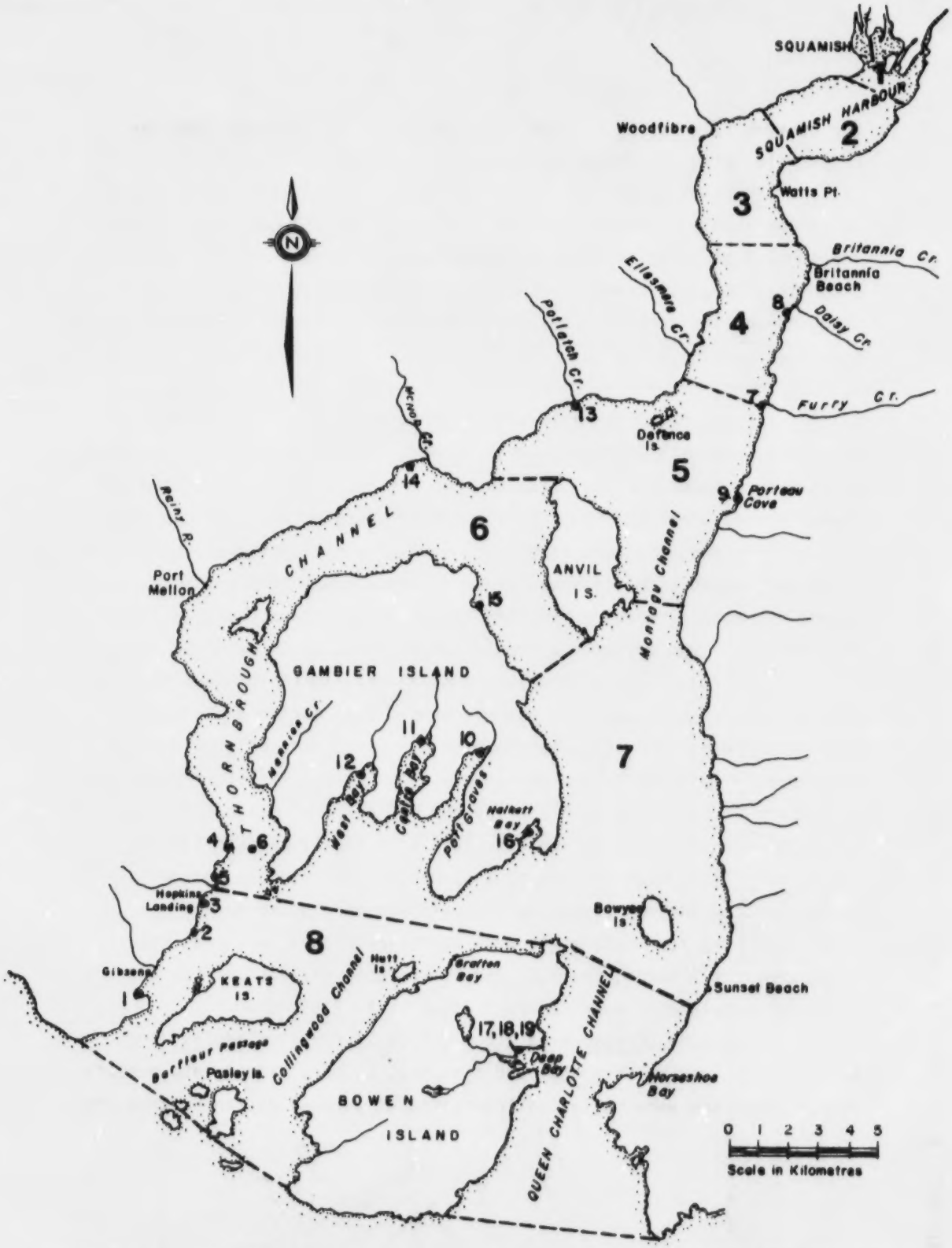

FIGURE 6 SHELLFISH SAMPLE LOCATIONS FOR TRACE METALS IN HOWE SOUND - June, 1981 (EPS)

the other metals may be related to copper and zinc tailings from the copper-zinc mines at Britannia Beach.

A study of mercury in the sediments at the upper part of the Sound compared sedimentary mercury levels for 1972-73 and 1976-77 (172). There was a decrease in mercury levels adjacent to the dock of the chloralkali plant. Levels in the sediments in a 15.5 km^2 area near the plant showed the same mercury levels for the two periods. The area tested comprises zones 1,2 and 3.

3.1.2 Heavy Metal Contamination from Pulp Mills. Problems of kraft and pulp mill effluents entering B.C. coastal waters were reviewed by Waldichuk some time ago (173) and more recently at the Symposium on Fisheries and Oceanography of the Strait of Georgia (15). At one time both mercury and zinc were associated with pulp mill effluents. The use of mercury-based slimicides was stopped around 1960. However a certain amount of residual mercury remained in the sediments near pulp and paper mill outfalls, and may pose a problem where dredging is required (15).

Zinc, as zinc dithionite (zinc hydrosulphite in the trade) was used as a brightener until it was discovered that marine organisms, especially oysters, were accumulating high concentrations of zinc. In 1973 the industry switched to sodium hydrosulphite (15). A twofold study was instituted at that time by Fisheries and Marine Service and EPS. Its purposes were (1) to establish zinc levels in shellfish near the four mills; and (2) to learn if boron, a by-product released as sodium metaborate, by the new bleaching process, was harmful to marine organisms. The four pulp mills were MacMillan Bloedel mills at Powell River and Port Alberni, the B.C. Forest Products mill at Crofton and the Crown Zellerbach mill near Campbell River (174, 175).

Oysters, (Crassostrea gigas), mussels (Mytilus edulis) and several clam species were analyzed for zinc, copper, cadmium and mercury. Sample organisms were collected from sites at various distances from the

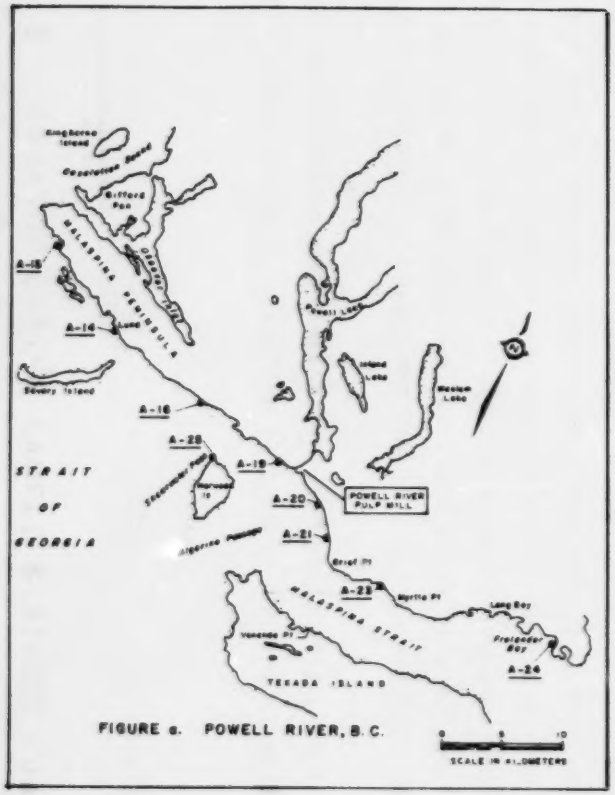

FIGURE a. POWELL RIVER, B.C.

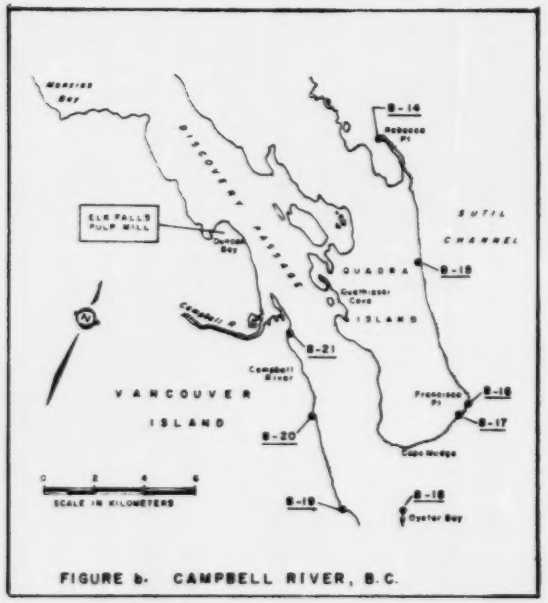

FIGURE b. CAMPBELL RIVER, B.C.

FIGURE c. CROFTON, B.C.

FIGURE 7 SHELLFISH STATION LOCATIONS NEAR THREE B.C. PULP MILLS (175)

mills as shown in Figure 7 for the mills as Powell River, Campbell River and Crofton. Others were collected at control stations, totally removed from mill effluent influence. Besides the metal analyses, oysters were examined for 'condition factor' (C.F.) in one collection. C.F., a measure of the general state of the oyster, is determined by body size. Values usually range from 40 (very thin oysters) to 150 (very plump) (175).

The smallest C.F. values were found at two of the control sites. Those closest to the mills had the next lowest values. It is therefore not possible to relate the C.F. values to mill effluent from these data. The results of the metal analyses are given in Appendix II, Table 5. Whereas zinc concentrations were in some cases very high, e.g. 17,280 $ug.g^{-1}$ dry wt. in oysters near the Powell River mill, levels of copper, cadmium and mercury did not seem to be influenced by proximity to the mills. Zinc concentration decreased with distance away from the mill. The influence of water mixing and currents is evident in the values from Powell River, where the highest zinc values occur at Station A-19, almost two miles west of the mill, whereas at Station A-20, about one mile east, the concentrations are less than half. The authors point out that the prevailing north-west tidal flow accounts for the higher values at station A-19.

Results of the EPS surveillance report (1977) (177) of the Crofton Pulp Mill effluent impact on oysters is given in Table 6, Appendix II. Mercury, zinc and cadmium were measured at ten stations in the "zone of influence" of the mill between January 19 and February 1. Boron was measured (at three stations) in oysters and mussels. PCBs were measured in oysters at eight stations, and were below detection in all cases. The location of the mill and the sampling stations are shown in Figure 8.

A long term study has investigated the recovery from zinc contamination in a stock of pacific oysters at Crofton (178). The

FIGURE 8 CROFTON OYSTER SAMPLE STATIONS - January 1977 (177)

oysters reduced their zinc load from 14.4 $mg.g^{-1}$ (dry tissue) in 1973 (when the discharge ceased) to 5.7 $mg.g^{-1}$ in 1979. The average concentration of zinc in control oysters, for the same period of time was 2.76 $mg.g^{-1}$. The condition factor averaged 25 in the contaminated oysters and 150 in controls. The level for marketability is 70. The authors found the condition factor had little predictive value over the years. They state that in the years since the discharge started there has been substantially lower condition factor in oysters near the outfall than in the control, indicating persistent contamination, but data since the zinc was stopped indicate it is not the cause of poor condition factor. The mean zinc values in test and control organisms, and the condition factors are given in Table 7, Appendix II.

3.1.3 Heavy Metal Contamination from Mines. At present three mines dispose of tailings into B.C. coastal waters: Island Copper Mine, on the north central shore of Rupert Inlet, Vancouver Island; Wesfrob Mines, on Tasu Inlet, Queen Charlotte Islands; and Amax Mines, at Kitsault, Alice Arm, B.C. Wesfrob and Island Copper mines began operations in 1970 and 1971 respectively. Amax began operation on April 1, 1981. Four other B.C. mines which also released tailings into marine waters have closed. All are listed in Table 8 (181). Collections of invertebrates for metal analyses, including bivalves, have been made at all three sites, to a greater or lesser degree, depending on the extent of the investigation.

3.1.3.1 Alice Arm - Hastings Arm. The Amax mine, located at Kitsault in Alice Arm, began operation in April 1981. Previously the site had been operated by B.C. Molybdenum from 1967 to 1972. In preparation for the reopening of the mine, EPS conducted studies to obtain baseline information prior to start-up and to assess the effects from the

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[1]editor's note: The Wesfrob and Amax mines ceased operations in October, 1983 and November 1982, respectively.

TABLE 8: MINING OPERATIONS IN B.C. INVOLVING SEA DISPOSAL OF TAILINGS (181)

MINE	LOCATION	METALS MINED	PERIOD OF OPERATION
Anaconda	Britannia Beach, Howe Sound	Copper, zinc	1900-1975
Anyox	Anyox, Hastings Arm	Copper	1914-1936
B.C. Molybdenum Ltd.	Kitsault, Alice Arm	Molybdenum	1966-1972
Jordan River Mine	Jordan River, Vancouver Island	Copper	1960-1974
Amax Canada Ltd. (formerly Climax Molybdenum of BC)	Kitsault, Alice Arm	Molybdenum	1981-1982
Wesfrob Mines	Tasu Inlet, Queen Charlotte Islands	Copper-zinc	1970-1983
Island Copper Mine	Rupert Inlet	Copper-Molybdenum	1971-

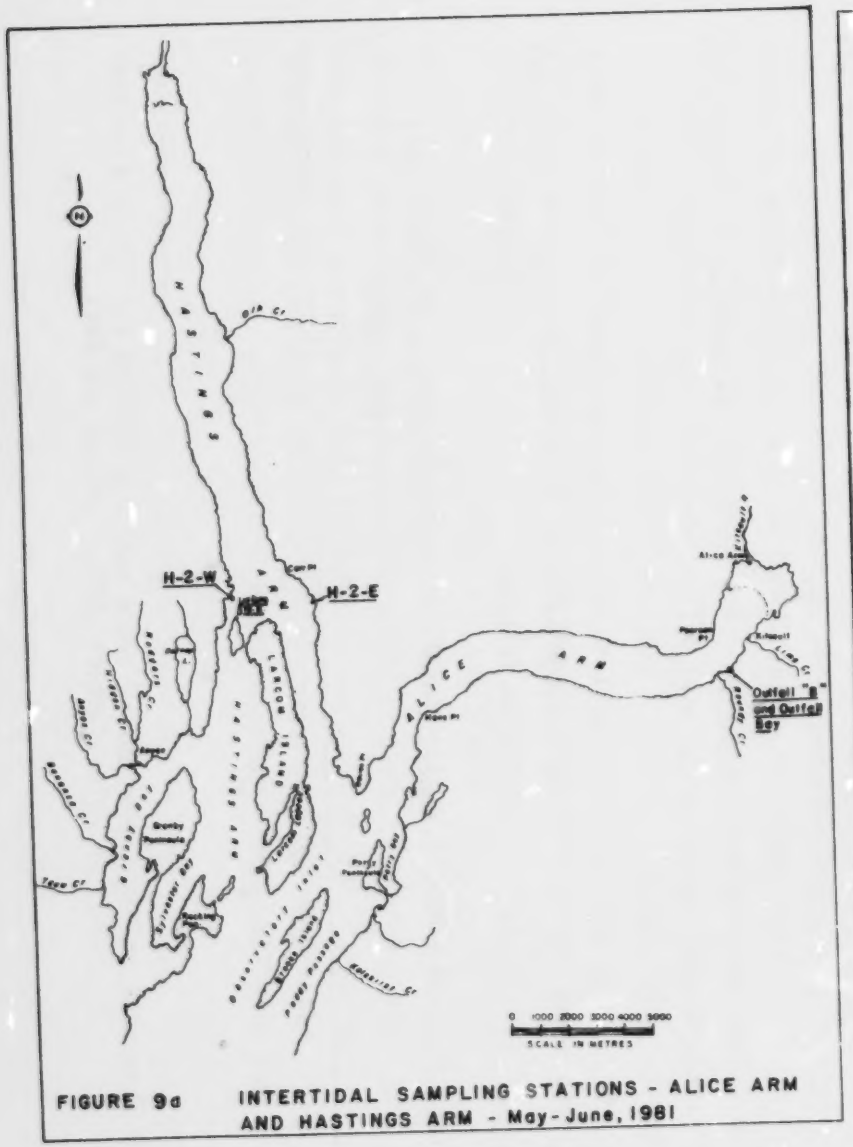

FIGURE 9a INTERTIDAL SAMPLING STATIONS - ALICE ARM AND HASTINGS ARM - May-June, 1981

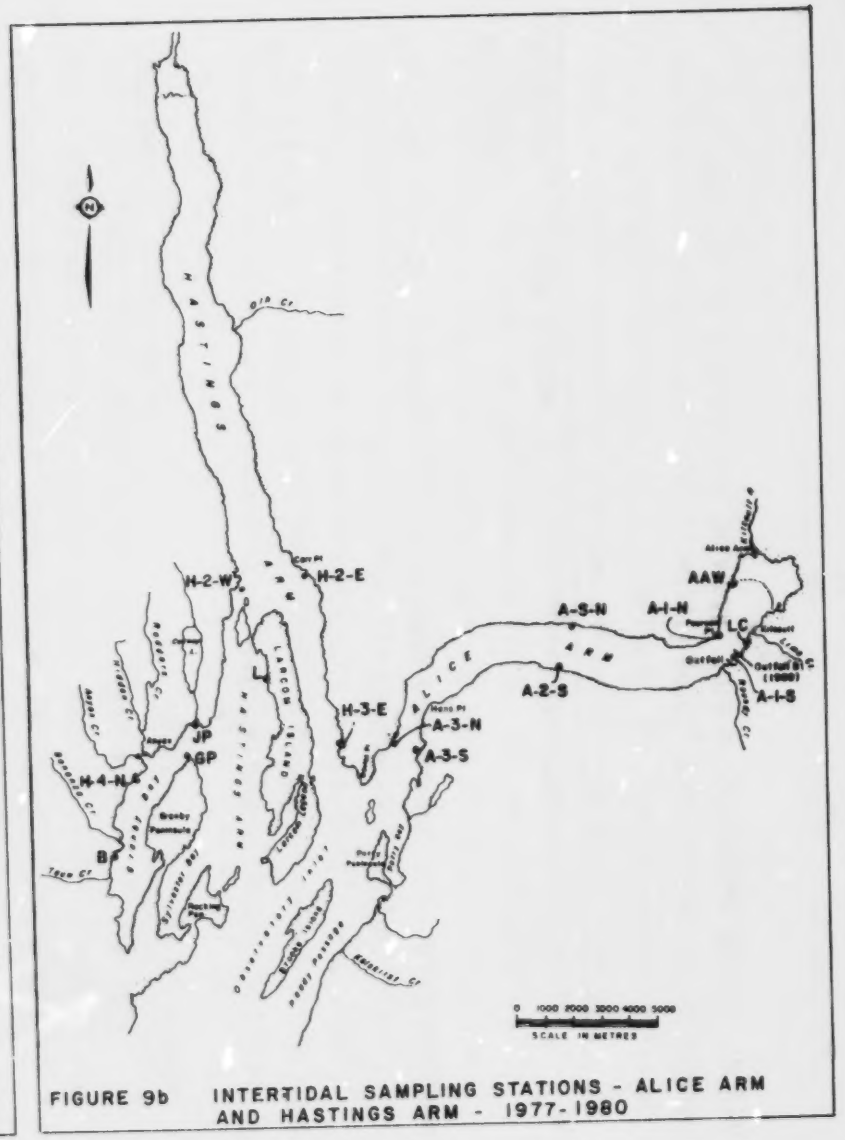

FIGURE 9b INTERTIDAL SAMPLING STATIONS - ALICE ARM AND HASTINGS ARM - 1977-1980

FIGURE 9 INTERTIDAL SAMPLING STATIONS - ALICE ARM AND HASTINGS ARM (179)

previous B.C. Molybdenum operation and a nearby abandoned copper smelter at Anyox. These studies were undertaken 1) between 1976 and 1980; 2) immediately following start-up in April 1981 and 3) periodically throughout the initial production period. Mussel samples (M. edulis) were collected at a variety of sites, shown in Figure 9. Trace metal tissue analysis summarized by Goyette and Christie (179) is presented in Appendix II, Table 8. The data accumulated during the seven months following start-up of the mine showed that tissue trace metal concentrations in most species sampled had not changed significantly from data collected during or prior to start-up (179). Comparative data for M. edulis was available for only 2 sites in Hastings Arm and showed some increase in Cu, Pb, Cd and Fe levels.

A study by a consulting company from 1974-77, included analysis of copper, zinc, lead, cadmium and molybdenum, in twelve organisms, including mussels and cockles (180). The combined results of analyses taken in three years are given in Table 9, Appendix II.

3.1.3.2 Quatsino Sound. A monitoring program has been conducted at the Island Copper Mine site by Utah Mines Ltd., as a condition of the permit to discharge mine tailings into Rupert Inlet (181). During the early years, EPS conducted a surveillance and monitoring program to assess the behaviour of wastes in relation to the company's pre- operational predictions (182). Part of the program involved regular sampling of bivalves at a number of stations in Rupert Inlet, Holberg Inlet, Quatsino Narrows and the eastern end of Quatsino Sound. From 1971-74, the mussel (M. edulis) and four clams (Macoma iris, Mya arenaria, Saxidomus giganteus and Protothaca staminea) were collected and analyzed for zinc and copper. These data are presented in Table 10, Appendix II. The collecting sites for the first four years of the programme are shown in Figure 10. Figure 11 shows the company monitoring sites up to the present. Since 1975 seven metals, including zinc and copper, have been routinely analyzed.

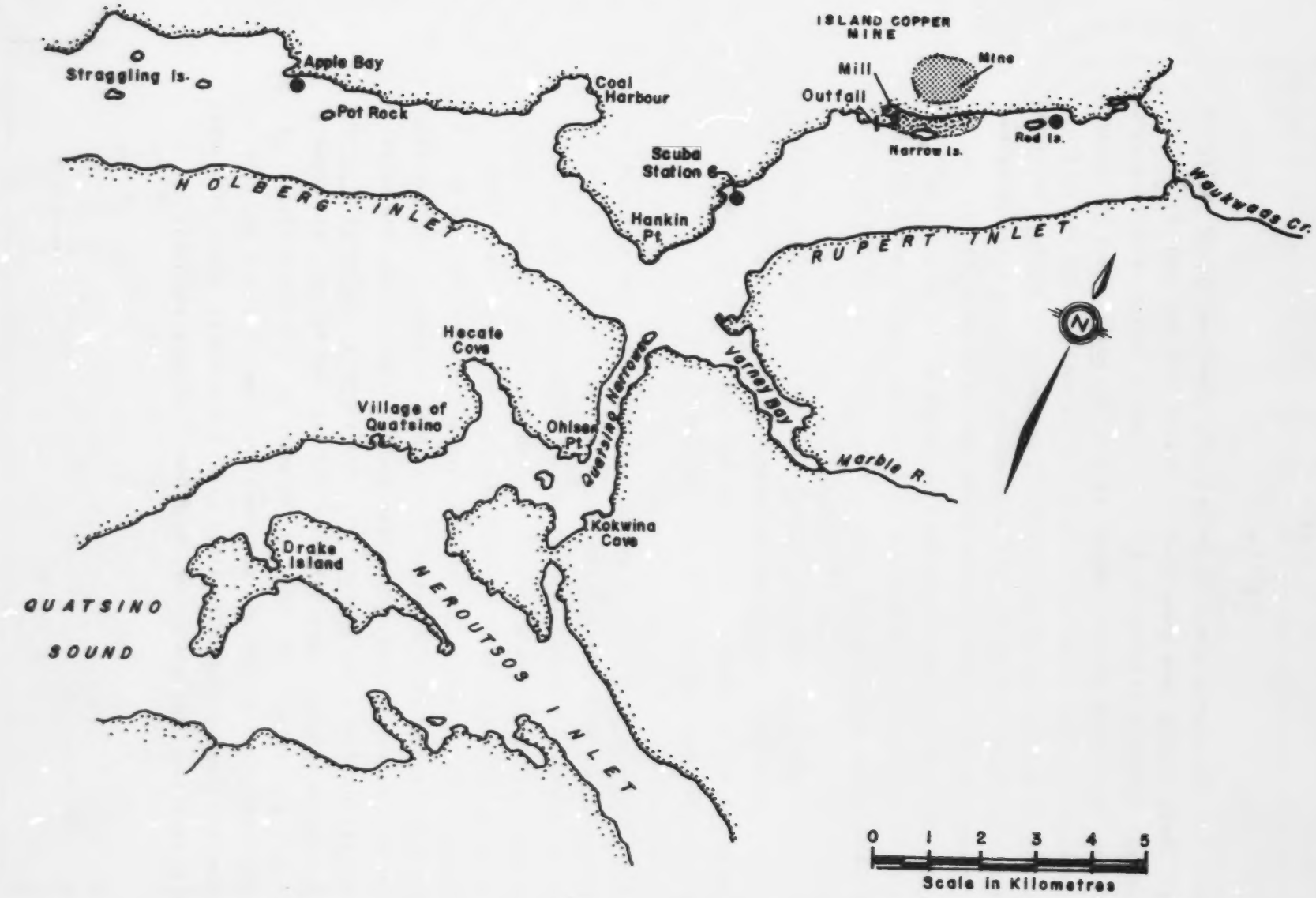

FIGURE 10 SHELLFISH SAMPLE LOCATIONS FOR TRACE METALS, ISLAND COPPER MINE - 1971 - 1974 (EPS) (182)

FIGURE 11 SHELLFISH SAMPLE LOCATIONS FOR TRACE METALS, ISLAND COPPER MINE - 1971-1978 (Company Sites) (183)

In the 1978 Annual Environmental Assessment Report (183), a statistical comparison was made of selected metals (copper, zinc and arsenic) in the intertidal clams, M. arenaria, P. staminea, and the mussel, M. edulis. Data for each species was pooled for the periods June 1971 - Sept. 1972, and Dec. 1976 - Sept. 1978 (Table 11, Appendix II). Statistical analyses (ANOVA and Newman-Keul multiple range test) showed some significance between stations, especially for copper among both clamspecies, but not a clear trend over the total time span. For example, in P. staminea values for copper (ppm) ranged between 0.94 and 2.32 at the five stations in 1971-72, and were 0.97 to 1.87 in the 1976-78 period. Intermittent changes in copper and zinc values at stations R4 and R6 are attributed to mining operations, since they are close to the mine (183). At the mussel stations, the highest values for copper and zinc were found at the Utah dock. The highest copper values (3.5 and 4.7 ppm wet wt.) occurred in March of 1978 and 1979 respectively. The highest zinc values were in December, 1979 (47 ppm wet wt.) and June 1977 (43.03). It is assumed that the "slightly elevated copper and zinc levels in the tissues of blue mussels collected from the Utah dock were probably due to the 2 x 10^4 tons of copper concentrate (typically 23% copper and 0.2% zinc) loaded .... on a monthly basis" (183). The copper levels at the other two docks were not above 2.7 ppm, with an average of about 1.5 ppm.

Deep water clams, Humilaria kennerleyi, were sampled sufficiently at Hankin Point to allow detailed statistical analysis. Between April 1976 and September 1978, there were no detectable increases in copper, zinc or arsenic levels (Table 12, Appendix II). The first two metals were at the lowest concentration for any organism monitored, whereas arsenic levels were among the highest (exceeded only by Cancer magister). This station is approximately 5.5 km from the mine outfall.

Data for all seven metals in the mussels at the three stations closest to the three loading docks are given in Tables 13, Appendix II.

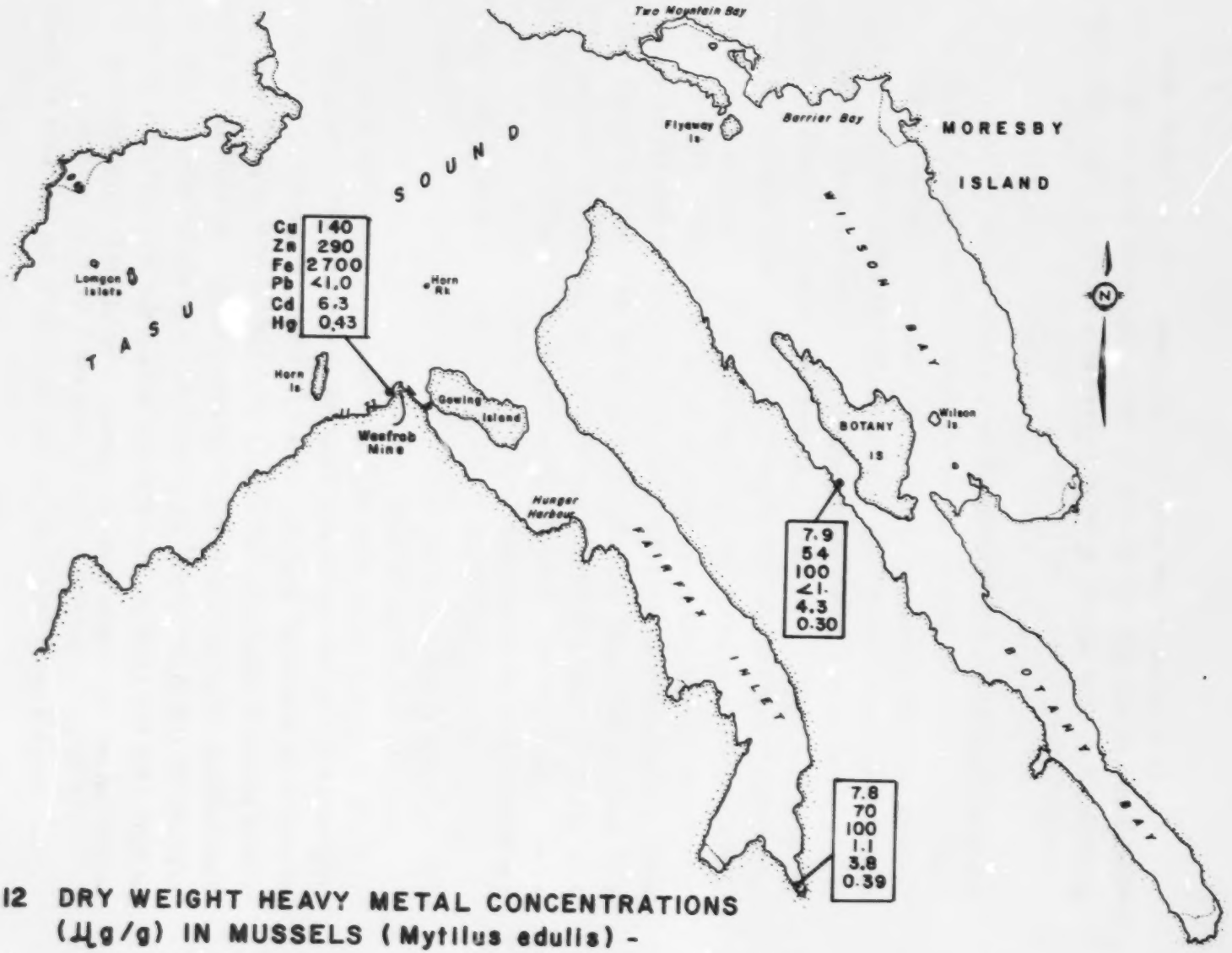

FIGURE 12 DRY WEIGHT HEAVY METAL CONCENTRATIONS (μg/g) IN MUSSELS (*Mytilus edulis*) - WESFROB MINE - June 1977 (184)

In a review of the monitoring programme at Island Copper Mine, recommendation was made for monitoring "bioaccumulation by a standard organism, e.g. blue mussel, Mytilus edulis, at a number of representative locations" (181).

3.1.3.3 Tasu Sound. EPS conducted a survey in 1977 to assess the impact of the Wesfrob mine on the marine receiving environment. Raw tailings had been discharged by this mine since 1967. The survey was scheduled to "coincide with the termination of the sinter circuit tailings discharge to the foreshore" and diversion to a submarine outfall (184). The mussel, M. edulis, was sampled as part of "intertidal observations and collections", and analyzed for copper, zinc, iron, lead, cadmium and mercury. Metal concentrations and site locations are depicted in Figure 12. Copper concentrations range from 140 $ug.g^{-1}$ (dry wt.) close to the mine, to 7.8 $ug.g^{-1}$ at a control station at the head of Fairfax Inlet. Zinc and iron were also much higher at the mine site, 290 ppm (zinc) and 2700 ppm (Fe), compared with 70 ppm (Zn) and 100 ppm (Fe) at the control sites. Levels of lead, cadmium and mercury were not appreciably greater at the mine sites than at the control sites (184).

3.2 PAH in B.C. Bivalves

PAH were first reported in B.C. shellfish by Dunn and Stich (131) in an investigation of mussels as bioassay organisms for B(a)P (2). They found 0.2 $ug.kg^{-1}$ in mussels from the open west coast of Vancouver Island, 5 km from human activity; 42.8 $ug.kg^{-1}$ B(a)P were found in mussels from a poorly flushed inlet with heavy boat and industrial use. There were some seasonal fluctuations in B(a)P uptake by mussels at four out of five stations in Vancouver harbour, with a tendency to higher contamination in winter. The two outer harbour stations showed the greatest difference with winter values five times those of summer. The authors state that the

degree and pattern of seasonal fluctuations varied even though the stations were quite close to one another. They suggest the differences are caused by variations in pollution patterns, rather than by physical differences such as temperature , or physiological differences related to the breeding cycle. A possible local source is storm water which brings oil residues from the streets into the harbour. (Analysis of used crankcase oil showed 22,000 ug B(a)P.kg^{-1}, B.P. Dunn (185)).

Sediment samples were collected from the vicinity of Iona sewage treatment plant and analyzed for B(a)P. Concentrations were found to decrease with distance from the sewage outfall (186). Sewage treatment plants have actually been shown to reduce concentrations of PAH. However, during rainstorms, overflow discharges are flushed directly into Vancouver Harbour without treatment (186).

B(a)P was measured in commercial shellfish from seven B.C. locations, with oysters, collected at five of the stations, butterclams from the sixth, and geoducks from the seventh. The tissue was analyzed for B(a)P and six other related PAH compounds, as shown in Appendix III. The values are below those reported by Dunn & Fee for Canadian oysters (186). The highest value was 0.9 ng.g^{-1} wet wt. in geoducks at Courtenay area, with oysters at Denman Island at 0.8 ng.g^{-1}.

3.3 Chlorinated Organics in B.C. Bivalves

The report on toxic organic contaminants, a part of the Fraser River Estuary Study (16), included a broad range of industrially important organic compounds, identified by Health and Welfare Canada and Environment Canada as priority contaminants requiring further study. The report states that existing data on organic contaminants in the Fraser River System are primarily PCBs and organochlorine pesticides. This report includes only PCBs in relation to bivalves.

Two studies in 1972 analyzed PCBs and other chlorinated hydrocarbons in bivalves and other organisms in the Fraser River Estuary (187).

The highest PCB values were found in the oyster Crassostrea gigas with a value of 2.72 ppm dry wt. in the Roberts Bank region. The lowest value for oyster was 0.32 ppm dry wt. at Sturgeon Bank. Values for other mussels were in trace amounts or were non-detectable.

Data for PCBs found in bivalves from 1977 to the present are shown in Appendix IV (168). Levels in clams at Sturgeon Bank, measured in 1978 were lower than those at Roberts Bank. The highest were those measured in November at 76 $ug.kg^{-1}$ wet wt. Whereas the highest level at Sturgeon Bank was 0.3 $ug.kg^{-1}$ wet wt.

The highest levels in the province were found in mussels at Porpoise Harbour, in the Prince Rupert area. In June 1977, levels reached 17,000 $ug.kg^{-1}$ wet wt. measured in a composite mussel sample in the area of a PCB spill. Levels of 1700 $ug.kg^{-1}$ were measured at Lime Creek, in the Alice Arm area in February, 1981, also in the vicinity of a spill.

Additional data on chlorinated organics is presented separately in Section 3.5, Fraser River Estuary Pollutants.

3.4 Municipal and Storm Outfalls

Limited data has been collected for contaminants in bivalve molluscs in the vicinity of sewage and storm discharges. Those studies which have included bivalves in their monitoring programs are discussed separately in the following section.

3.4.1 McMicking Pt. Outfall. Trace metals and a number of organochemicals were analyzed in mussel tissue (Mytilus californianus) at varying distances from the McMicking Point outfall, and in M. edulis at the control site (188). Levels of copper, cadmium, lead and zinc are presented in Table 1, Appendix V. There was no significant difference between the experimental and control organisms, and the control animals had higher concentrations of copper and zinc. Mercury values are given in Table 2, Appendix V, an all values are quite low. Low levels of Aroclor 1254 and

traces of Aroclor 1242 were found in mussels near the outfall (Table 3, Appendix V). Aroclor 1254 was also found in mussels at the control site at even lower levels (84 ppb vs. 32 ppb at the two sites). A summary of the study indicates that the impact of the existing sewage discharge can be detected readily about 15 metres from the outfall, but is barely detectable at a distance of 50 to 100 m.

3.4.2 Comox (Goose Spit) Outfall. Analyses for five metals, copper, cadmium, lead, zinc and mercury were made on oysters from three locations, just north of the Comox municipal outfall. As it turned out, all values were at a level to be expected in such an area, as shown in Table 4, Appendix V. However in the first analysis high levels of mercury were found at Goose Spit (2.3 ppm wet wt.). Concern about the presence of this much mercury in an oyster growing area, among other uses, resulted in tests being made by four labs in toto. It appeared that high mercury levels were from contamination of the samples during preparation by one laboratory or possibly contamination from materials attached to the sample organisms in transit (189).

3.4.3 Other Studies. The mussel, Mytilus edulis was demonstrated as a "point source" indicator of metal pollution in Vancouver Harbour. X-ray energy spectroscopy was used to measure trace metal concentrations for lead, copper and zinc (68). The stations included a region close to a storm water outfall. The mussels were sampled at intervals of 1 km or less. "Dramatic" concentration variations were found, with mussels only 30 m from a point source of lead displaying essentially base-line concentrations (68). In a year-long study where metal levels were measured in mussels and in the seawater, it was found that for the most part concentrations in the mussels reflected those in the water. Copper was the one variant. Seasonal variations occurred in the lead, zinc, copper and iron in mussel tissue, but only occurred for soluble iron and zinc in the seawater.

A preliminary study was made by Bourne in Ladysmith Harbour (190) an area which had been, in the past, one of finest oyster growing areas in Georgia Strait. The results of his study indicate a decline in water quality in the harbour which may affect oyster breeding. More extensive investigations to distinguish between the sources and pollutants is ongoing by two consulting companies, EPS and the B.C. Ministry of Environment.

3.5 Fraser River Estuary Pollutants

The Fraser River has a profound effect on the southern waters of Georgia Strait. Its influence is discussed in a review of the physical oceanography of the Strait by LeBlond (191). An extensive study of the Fraser River Estuary was undertaken by Federal and Provincial government agencies to develop a management plan of the area. The study examined land use, recreation, habitat and water quality in the area downstream from Kanaka Creek to Roberts and Sturgeon Banks, as well as Boundary Bay and Semiahmoo Bay and the area between Point Grey and the U.S. Border. Burrard Inlet is not included.

Eleven background technical reports have been published, related to water quality (24). One of them, "Aquatic Biota and Sediments" covers estuarine organisms specifically (192). Other topics relevant to chemical pollutants and bivalves are: Water Chemistry (25), Toxic Organic Contaminants (16). Bivalves were not used as indicator organisms exclusively, but reference is made to them in several reports. The next section incorporates information on toxic pollutants in relation to bivalves in the Fraser River Estuary from the above-mentioned studies and from others.

3.5.1 Sewage Treatment Plants (STP).

Three large municipal sewage treatment plants at Iona, Annacis and Lulu islands, are considered to be the sources of greatest metal impact on the river, as well as other chemical pollutants (25). Stormwater discharges account for a large proportion of metals entering the river, with estimates of 65% of the

total iron and about 60% of total lead and nickel (193). Between 14 and 15% of the total copper and zinc loadings were estimated to come from stormwater discharges.

Industrial effluents are discussed in detail by Swain (193) and summarized by Stancil (192) in relation to estuarine biota. Industry contributes less than 10% of the total load of iron, lead, copper and nickel, but accounts for about 50% of the zinc load. Comparisons of individual metal contributions between industrial and other discharges are given by Swain (193) and Stancil (192). Landfill leachates are another source of metal pollution into the Fraser River. In general, metal contributions from landfills were less than from storm discharges or other effluents (194).

Bivalves sampled from the Sturgeon and Robert Banks in the Fraser River study were divided into two groups: those attached to jetties (oysters) and those which live in or on the sediments (clams). Organisms living on the jetties filter material from the water column, while those in sediments obtain food either from overlying water or from the sediments (192). Metals reach the organisms as dissolved or particulate matter in the water or in the sediments, interchanging from one to the other (as discussed in Section 2.1.1, and by Stancil with reference to the Fraser River).

Metal concentrations in organisms from the Iona Island jetty showed little difference between the south side of the jetty, influenced by the sewage outfall, and other stations, although concentrations of iron and lead were higher in oysters from the south side (Table 1, Appendix VI). The oysters do show much greater levels of cadmium, copper and zinc than do the clams, and to a lesser degree silver and manganese. The clams show higher levels of nickel. These differences may reflect variability rates of uptake between organisms, rather than differences of concentration of available metals at two different sites.

In 1976 the Environmental Protection Service monitored metal levels in soft tissues of burrowing bivalves from the sediments of Sturgeon

and Roberts Bank, as depicted in Figure 13. Four bivalves were collected for metal analyses, Macoma balthica, Cryptomya californa, Mya arenaria and Clinocardium nutalli. The collecting sites were grouped into four areas: (A) north of the Iona jetty; (B) south of the jetty (influenced by Iona STP); (C) south Sturgeon Bank; (D) Roberts Bank. The metal concentrations data, excluding mercury, were pooled from three clams (M. balthica, M. arenaria and C. californica) in the four regions (Table 2, Appendix VI). Cadmium, copper and to a lesser extent lead, appear to be more concentrated in specimens from the area affected by the sewage outfall. However the differences do not appear to be significant when the standard deviations are considered. Mercury data is given in Table 3, Appendix VI for Macoma sp. at all stations. Although there were significant differences in concentrations among the stations, as indicated by the Newman-Keul range test, significant differences did not exist among specimens taken from the north or south side of Iona jetty and Roberts Bank (192).

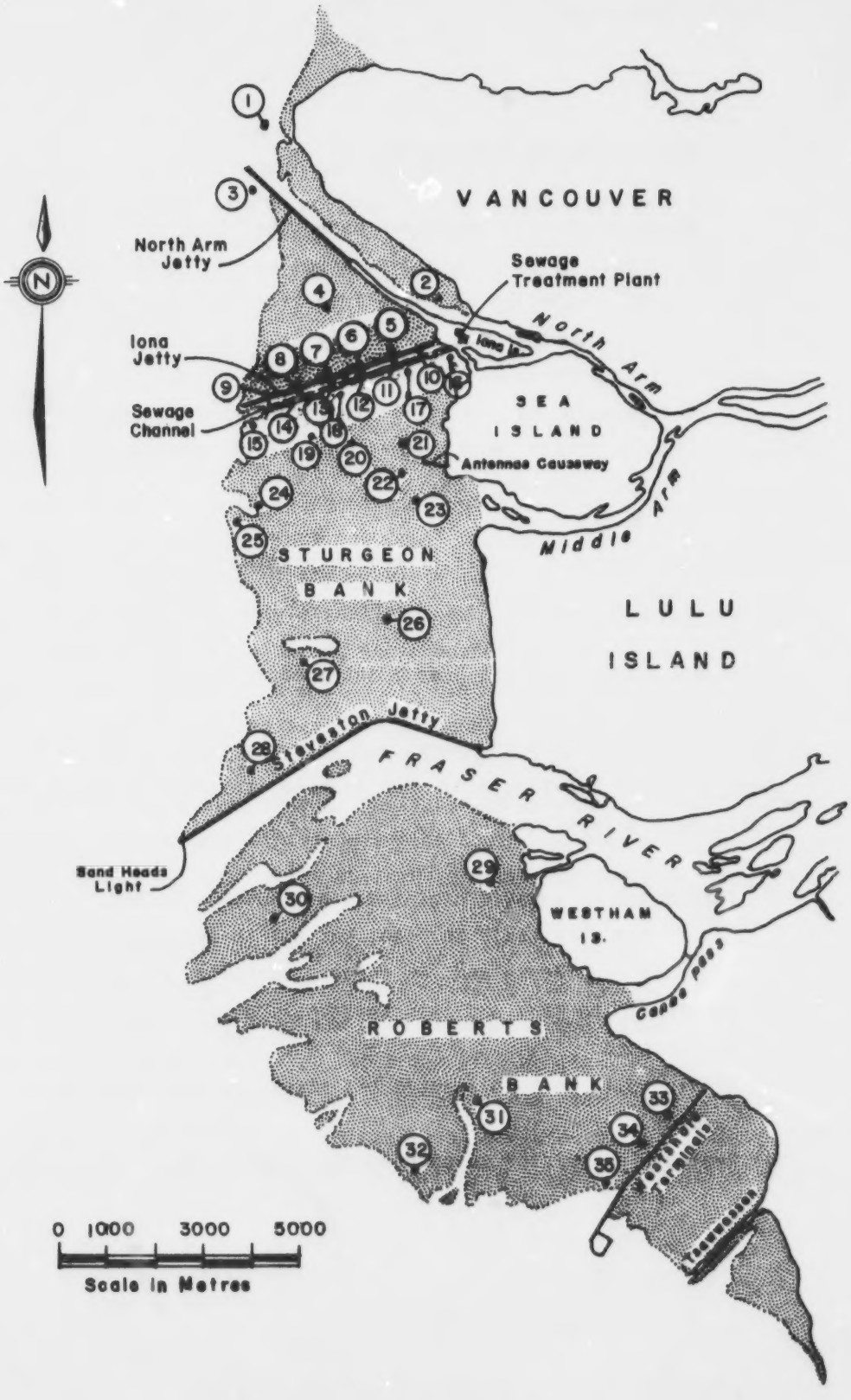

FIGURE 13 COLLECTION STATIONS FOR BIVALVES - STURGEON BANK AND ROBERTS BANK, 1976 (192)

4 DISCUSSION AND CONCLUSIONS

Numerous studies have been undertaken in British Columbia to monitor the effects of pollutant discharges on marine environmental quality. A good proportion of these investigations have utlized bivalve moîluscs as a measurement tool, primarily to assess the distribution of contaminants in a particular receiving water.

A discussion of the available information on toxic chemicals in coastal waters and their relationship to bivalves must be prefaced with a recommendation for awareness that:

(a) the significance of levels of most contaminants and pollutants in the environment is not known;

(b) many pollutants have not been identified (195);

(c) toxicity varies with organisms and with the form of the pollutant, best known in heavy metals. For example, toxicity of heavy metals falls into several groups:

i) Very toxic to aquatic organisms, and less so to man (e.g. zinc and copper are very toxic to fish, but not to people.) Such metals can have high acute and low chronic toxicity, or vice versa.

ii) Very toxic to man, and less to aquatic organisms, e.g. chromium and arsenic.

iii) Highly toxic to both aquatic organisms and man, e.g. mercury and cadmium.

iv) Negligible toxicity to both aquatic organisms and man, e.g. iron.

In turn aquatic organisms must be separated into sub-groups. Heavy metals which are harmful to fish can be accumulated by adult bivalves, without any obvious harmful effects. However, deleterious effects from long term, low level chronic insult to the adults may exist and are not understood. The more fragile life stages, e.g. eggs and embryos, are clearly susceptible to heavy metal pollution, as discussed in Section 2.1.4.

As Wood points out (5) mercury and other metals have a biological cycle, (as shown in Fig. 14). He suggests that the fate of mercury and the synthesis of various mercurial organic compounds by microorganisms may also be found with other metals. When 'natural' biological cycles are perturbed by additional sudden inputs of the metal in question (from anthropogenic sources, for example) the long-established equilibrium is shifted with unknown effects on the metal forms, their quantities and their position in the cycle. The metal states are also affected by pH, temperature, redox potential and possible synergistic and antagonistic effects with other metals. It is also important to know their solubility in water and in lipids. This is especially true of newly formed compounds. Their maximum concentration in water and lipids determine their "bioaccumulation potential", which can help decide their role as environmental contaminants (5).

Much of the information about toxic chemicals and marine bivalves is related to the use of several bivalve species as indicator or 'sentinel' organisms. Bivalves are used to monitor pollutants qualitatively and quantitatively. They may also be used, in certain cases, to identify the source of pollutants, as has been shown in many studies of heavy metals related to industrial pollutants, mentioned in Section 2.1.4. The absence of certain sensitive organisms can also be an

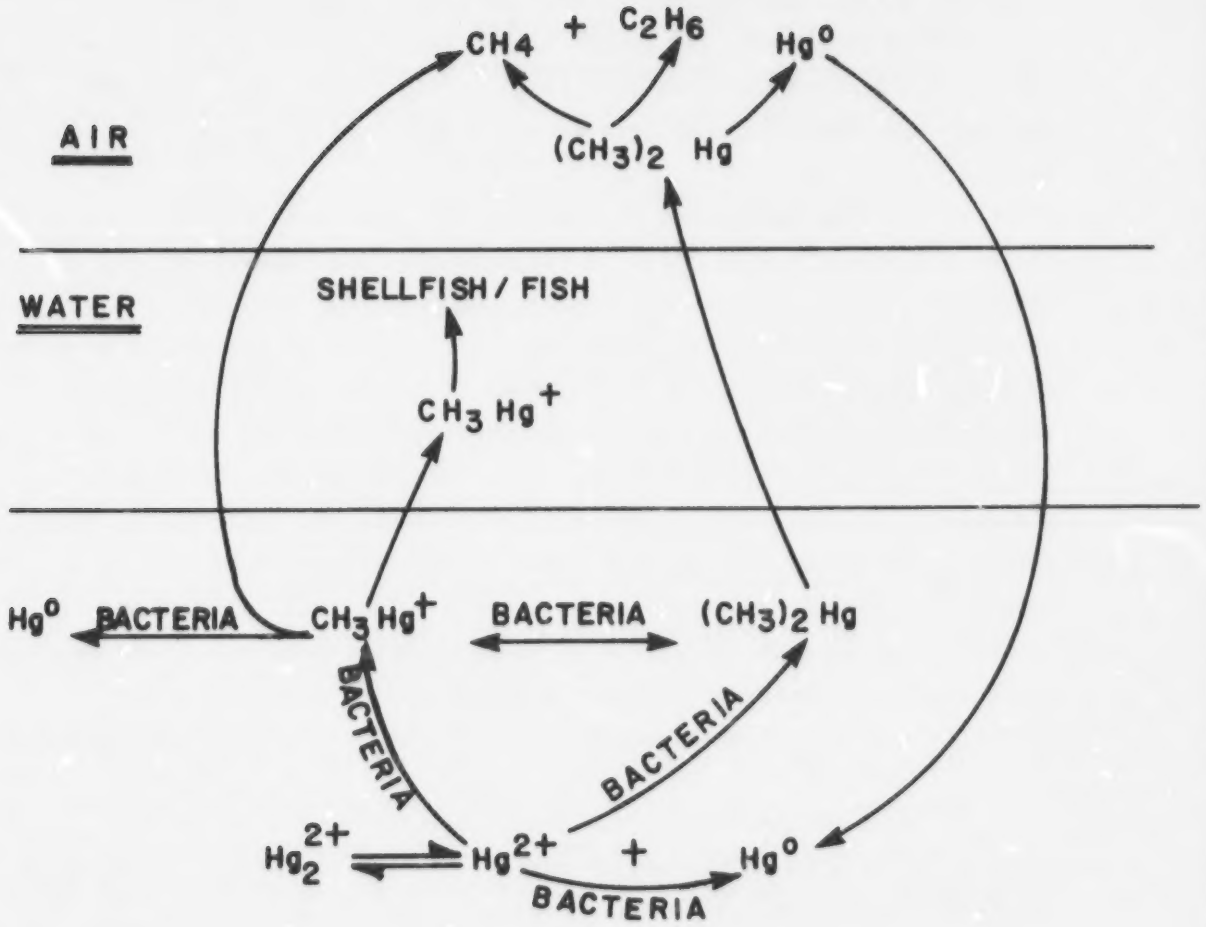

FIGURE 14 BIOLOGICAL CYCLE FOR MERCURY (5)

indication of pollution, although causative factor(s) cannot be identified. The practice of using bivalves as indicator organisms for monitoring marine pollution is comparatively new and methods are not refined enough to make valid comparisons of pollution levels from place to place. The results of the International Mussel watch conference (4) indicate that while mussels and other bivalves are becoming common 'sentinel' organisms, and offer many advantages over other marine organisms, they cannot be considered a panacea.

Filter feeding organisms can obtain and retain contaminants both dissolved in the water column and attached to particulate matter. They can thereby 'measure' quantities of a contaminant over a defined time period, depending upon the kinetics of uptake and release. The kinetics are dependent upon species, physiological state, age, season physical parameters and the form or state of the contaminant. Sediment-bound contaminants are measured in deposit feeders. If released by microbial or chemical activity, into the water column, such contaminants may be taken up by filter-feeding bivalves, but cannot be identified as having originated from the sediment. Changes in levels of pollutants over time can be monitored by transplanting appropriate bivalves from a 'clean' area to the suspected area, as described in Section 2.1.4.

Less is known about the fate of organochemicals in the marine environment than of heavy metals. With new substances entering the atmosphere and the oceans in increasing numbers, keeping track of those which are toxic and dangerous presents an added difficult problem. For example, toxaphene is considered to be an especially dangerous compound. It is chlorinated camphene, used for the control of grasshoppers and other pests, and a known carcinogen, from tests with rats and mice. Yet it was able to be identified by only a few laboratories in a test of pesticide residues (195).

British Columbia is in the enviable position of having a comparatively unpolluted marine environment, as indicated in Section 3 and in the recent review of pollution in Georgia Strait by Waldichuk (15).

The majority of the studies outlined in Section 3 were monitoring programs conducted in response to known pollutant hazards rather than surveillance of bivalve growing waters. Because of their nature and purpose they were carried out independently by a number of different investigators. Although they all included bivalves as test organisms, the data are not directly comparable, but are summarized in Table 9 for information purposes.

Conclusions regarding toxic chemical pollution of bivalves in B.C. coastal waters can be summarized as follows:

1) A number of studies indicated pollution effects on bivalves from sewage treatment plants, including heavy metals such as mercury, lead and cadmium. These metals enter the marine environment from other sources as well. The Fraser River Estuary Study, and others, pointed out the negligible growth of bivalves and other marine organisms close to the outfalls.

2) Organochlorines, notably PCBs, DDT and its breakdown products, have been detected in local waters, and in organisms, but not at dangerous levels.

3) PAH, represented by Benzo(a)pyrene, have been analyzed in sediments in the Fraser River Estuary, areas of Vancouver Harbour and in commercially harvested bivalves. It is suggested storm drains are the source of much of PAH contamination, although contamination is also associated with creosoted wood pilings.

TABLE 9: SUMMARY OF TRACE METAL, PCB AND PAH LEVELS IN B.C. SHELLFISH

	ppm					
	Hg		Cd		Pb	
SPECIES	wet wt.	dry wt.	wet wt.	dry wt.	wet wt.	dry wt.
M. edulis	0.004-0.042	< 0.088-0.43	0.24-1.3	1.6-9.46	< 0.1-1.6	< 1.0-44
C. gigas	0.02-0.15	0.22-0.59	0.55-3.4	2.42-26.7	0.17-0.6	0.66-12.3
S. giganteus	0.02		0.06	0.3	0.2	0.8
P. staminea	0.04-0.034		0.1-0.17	0.5-1.2	< 0.1-0.6	< 1.0-3.0
M. arenaria	0.027-0.032	0.37	0.088-1.50	< 1.0-10.6	< 0.531-1.74	2.0-5.24

	ppm				PCB	PAH
	Cu		Zn		$ug \cdot kg^{-1}$	$ng \cdot g^{-1}$
SPECIES	wet wt.	dry wt.	wet wt.	dry wt.	(wet wt.)	(wet wt.)
M. edulis	0.75-6.86	0.3-243	6.69-47	34-462	9.0-17,000[1]	
C. gigas	0.16-260	0.59-1210	19.5-5100	72-22,000	322.3-2724.1[2]	0.3-0.8
S. giganteus	< 1.3	11-38	15	59-162	0.05-87	0.4
P. staminea	0.97-2.7	9.2-40	8.3-16.9	60-137	0.05-87	
M. arenaria	0.73-10.4	16-77	8.0-48.6	62-304		

[1]high value of 17,000 resulted from a PCB spill
[2]dry weight

4) Pulp mills, mines and a chlor-alkali plant have been responsible for elevated heavy metal levels noted in shellfish. Changes in methods by pulp mills in a bleaching process, and in the use of mercury in the chlor-alkali plant have reduced the quantity of zinc and mercury respectively being introduced into the nearshore waters.

5) Municipal sewage outfalls can be significant sources of heavy metal and organochlorine pollutants, as well as bacteriological contamination. In some cases analyses are made of receiving water, and organisms, for toxic chemicals as well as coliform bacteria, as part of studies on the impact of an outfall, but

6) There is no systematic monitoring programme for toxic chemicals in the marine environment, equivalent to the bacteriological program.

7) In addition, although there is a good deal of scattered information related to toxic contaminants and bivalves in B.C. marine waters, there is none in which the growing waters in particular are considered. At present, elevated levels of contaminants have not been noted in bivalves from the major commercial harvesting areas in B.C.

The absence of high levels of contaminants in bivalve molluscs from major harvesting areas is encouraging although not totally unexpected. The majority of the province's shellfish growing sectors are located in areas of excellent water quality and there is minimum conflict with other users of the marine environment. However, industrial and urban development in British Columbia coastal areas will continued to encroach on some of the shellfish growing waters. It is therefore important to ensure programmes for the surveillance of water (and shellfish) quality for toxic chemicals

are developed, in addition to the bacteriological and sanitary programmes presently in place. Furthermore, consideration should be given to the development of longer term projects to relate the movement of contaminants and their uptake by bivalve molluscs and other organisms to the physical characteristics of coastal waters. There is abundant information on the physical oceanography of the coast, particularly Georgia Strait (reviewed by Leblond (191)). It would appear timely to integrate this knowledge with marine pollution monitoring studies in the development of better predictive tools.

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APPENDIX I

PHYSICO/CHEMICAL PROPERTIES OF LEAD, MERCURY AND CADMIUM

APPENDIX I PHYSICO/CHEMICAL PROPERTIES OF LEAD, MERCURY AND CADMIUM

LEAD

Lead, Pb; periodic system group IVB; atomic weight 207.19; atomic number 82; density 11.34; melting point 327.5°C; boiling point 1749°C; silver, bluish white soft metal; oxidation states 0, +2, +4. In most organic compounds, lead is in the +2 oxidation state. Lead oxides and lead sulfide are poorly soluble in water, while lead acetate, lead chlorate and to some extent lead chloride are soluble.

The most important organic/lead compounds are tetramethyllead, and tetraethyllead, because of their widespread use as anti-knock fuel additives. Both are colourless liquids with boiling points of 110°C and 200°C respectively. At these temperatures, or slightly below, they begin to decompose. About 10% of ingested lead is absorbed in the gastrointestinal tract, and the porportion may be higher in children. Lead body burden consists essentially of two compartments. Bone, with about 90% of the total content has a half-time of about 20 years, with the amount of lead increasing throughout life. Blood is the second, small compartment, with soft tissue and rapidly exchangeable bone fraction also contributing. The half-time in these compartments is about 20 days.

MERCURY

Mercury, Hg; occurs as elemental mercury, inorganic and organic compounds (mercury vapor, mercury liquid, mercury salts, short-chain alkylmercury compounds, alkoxyalkylmercury compounds and phenylmercury compounds) all with different toxicological properties.

Atomic weight, 200.6; atomic number 80; density 13.6; melting point -38.9°C; boiling point 356.6°C; crystalline form is silver white metallic liquid; oxidation states +1, +2. Metallic mercury is rather volatile. The vapor is regarded as insoluble in water, although at room temperature its solubility is approximately 60 mg/l. Solubilty in lipids

is 5-50 mg/l. It is rapidly oxidized by oxygen to ionic form. Mercuric salts, like halides sulfates and nitrates are water-soluble. An equilibrium is established between Hg^0, Hg_2^{2+} (mercurous) and Hg^{2+} (mercuric), depending on the redox potential of the solution and the presence of compounds which can form complexes with the mercuric ions. The mercuric ion can form many stable complexes with biologically important molecules or moities. Mercurious mercury is rather unstable in the presence of biological molecules. Volatile salts are formed at room temperature between short chain alkylmercuric compounds and halogens. Other salts, such as hydroxides and nitrates are less volatile.

CADMIUM

Cadmium, Cd; atomic weight 112.4; atomic number 48; density 8.6; melting point, 320.9°C; boiling point, 765°C; silver-white malleable metal; oxidation state, +2. Of the many inorganic cadmium compounds, e.g. cadmium acetate, cadmium carbonate, several are quite soluble in water, namely the acetate, chloride and sulfate. Cadmium oxide and sulfide are almost insoluble, in water. Cadmium is easily complexed with some organic compounds, e.g. thiocarbamates. There are some synthetic organo-metallic compounds, but they decompose rapidly and are therefore not found in the environment.

APPENDIX II

SUMMARY OF B.C. TRACE METAL DATA FOR BIVALVE MOLLUSCS

APPENDIX II

TABLE 1 TRACE METALS IN BIVALVES IN SOUTHERN B.C. WATERS (1971-1973)(170)

SITE Common Name (Species)	Date yr.	 mo.	(ppm) Hg wet	Cu wet	Cu dry	Zn wet	Zn dry	Pb wet	Pb dry	Cd wet	Cd dry	Ni wet	Ni dry
Port Hardy, Hardy Bay													
Butter clam													
(Saxidomus giganteus)	71	05	0.02	4.3	17	15	59	0.2	0.8	0.06	0.3	0.8	3.5
Alert Bay, Broughton Strait													
Blue mussel													
(Mytilus edulis)	73	02	0.066	2.2	0.3	29	170	0.2	1.0	1.1	7.9	--	--
Campbell River, Discovery Passage													
Pacific oyster													
(Crassostrea gigas)	71	06	0.04	210	1000	5100	22,000	--	--	--	--	--	--
" "	71	06	--	260	1200	4400	20,000	--	--	--	--	--	--
" "	72	10	0.05	16	76	490	2,300	0.2	1.0	1.9	8.7	--	--
Powell River, Harwood Vivian Islands													
Pacific oyster													
(C. gigas)	72	10	0.1	34	150	1800	8,000	0.2	1	2.0	8.9	--	--
Hornby Island, St. of Georgia													
Pacific oyster													
(C. gigas)	71	06	0.02	--	--	--	--	--	--	--	--	--	--
" "	72	10	0.15	23	93	450	1,800	0.6	2	1.4	5.6	--	--
Egmont													
(C. gigas)	71	05	0.04	58	230	670	2,700	0.22	0.91	3.4	14.0	--	--

Continued...

APPENDIX II

TABLE 1 TRACE METALS IN BIVALVES IN SOUTHERN B.C. WATERS (1971-1973) (170)
(Continued)

SITE Common Name (Species)	Date yr.	Date mo.	Hg (ppm) wet	Cu (ppm) wet	Cu (ppm) dry	Zn (ppm) wet	Zn (ppm) dry	Pb (ppm) wet	Pb (ppm) dry	Cd (ppm) wet	Cd (ppm) dry	Ni (ppm) wet	Ni (ppm) dry
Pender Harbour, Malaspina Strait													
Pacific oyster (C. gigas)	72	10	0.04	28	150	530	2,907	0.4	2	1.8	10	--	--
Mudge Island, Gabriola Island													
Littleneck clam (Protothaca staminea)	72	10 (5)	0.04	2.7	10	13	50	0.6	3	0.1	0.5	--	--
Pacific oyster (C. gigas)	72	10 (6)	0.06	6.6	25	310	1,300	0.5	2.8	0.8	3	--	--
Thetis Island, Stuart Channel													
Pacific oyster (C. gigas)	71	08	0.02	17	72	630	4,800	0.15	1.0	--	--	--	--
Chemainus - Crofton Stuart Channel													
Pacific oyster (C. gigas)	71	04	0.03	--	--	--	--	--	--	--	--	--	--
Stuart Island, Haro Strait													
Pacific oyster (C. gigas)	71	09	0.03	0.16	0.59	19.5	72	0.17	0.66	--	--	--	--

Continued...

APPENDIX II

TABLE 1 TRACE METALS IN BIVALVES IN SOUTHERN B.C. WATERS (1971-1973) (170)
(Continued)

SITE Common Name (Species)	Date yr.	Date mo.	Hg wet (ppm)	Cu wet (ppm)	Cu dry (ppm)	Zn wet (ppm)	Zn dry (ppm)	Pb wet (ppm)	Pb dry (ppm)	Cd wet (ppm)	Cd dry (ppm)	Ni wet (ppm)	Ni dry (ppm)
Saturan Island, Haro Strait													
Pacific oyster													
(C. gigas)	71	09	0.03	20	91	560	2,660	0.14	0.66	--	--	--	--
" "	72	10	0.03	23	120	500	2,500	0.6	3	--	--	--	--
Lower Indian arm, Port Moody													
Blue mussel													
(Mytilus edulis)	73	01	.13	1.4	11	14	118	1.4	11	0.3	2.4	--	--
Crescent Beach, White Rock													
Blue mussel													
(M. edulis)	73	01	0.039	1.8	12	16	100	0.2	1	0.2	2	--	--
Sooke, Jordan River													
Horse clam													
(Tresus capax)	71	09	0.02 (3)	3.3	17	8.4	43	0.15	0.5	0.06	0.33	--	--
Gold River, Muchalet Inlet													
Blue mussel													
(M. edulis)	72	10	0.06	1.3	8.0	12	76	--	--	0.5	3.0	--	--
Coal Harbour, Holberg Inlet													
Horse clam													
(T. capax)	71	10	0.02	--	--	--	--	--	--	--	--	--	--

APPENDIX II

TABLE 2 TRACE METALS IN BIVALVES IN SOUTHERN B.C. WATERS (1976-1977) (170)

From: "TRACE METALS IN MARINE ORGANISMS FROM COASTAL WATERS OF B.C."

Draft Report, Fisheries and Oceans, 1980, Harbo, R., Bi:twell, F.K., Langer, O.E. Heavy Metal Concentrations in Crassostrea gigas (Pacific Oyster)

DATE	LOCATION	ug.g^{-1} (wet/dry)				n
		Hg	Cd	Zn	Cu	
1976, June 5	Cortes Is.	0.040/----	----	----	----	10
1976, Dec. 14	Mudge Is. Gabriola Is.	0.09/----	1.44/6.25	393/1705	17.64/76.24	20
1977, Dec. 14	Mudge Is. Gabriola Is.	0.091/----	1.26/5.44	498/2100	16.70/70.2	10

APPENDIX II

TABLE 3 STATISTICAL COMPARISON OF MERCURY CONCENTRATIONS IN MUSSELS (*Mytilus edulis*) IN HOWE SOUND (1972-1975) (171)

a) by collection years

SAMPLING ZONE	YEAR AND MEAN MERCURY CONTENT ($ug \cdot g^{-1}$)				
All zones 1,4,5,6,7,8	Year	1975	1972	1973	1974
	Mean	0.07	0.07	0.06	0.06
Zones 1,4,5	Year	1975	1973	1974	
	Mean	0.08	0.05	0.05	
Zones 6,7,8	Year	1973	1972	1974	1975
	Mean	0.08	0.07	0.07	0.02

b) by collection zones

YEAR	SAMPLING ZONE AND MEAN MERCURY CONTENT ($ug \cdot g^{-1}$)						
1972 to 1975	Zone	1	8	6	7	5	4
	Mean	0.42	0.13	0.05	0.04	0.03	0.03
Grouped zone data	Zone	(1,4,5)		(6,7,8)			
	Mean	0.07		0.05			

*Solid lines under groups indicate homogenous subsets ($p < 0.05$).

APPENDIX II

TABLE 4 CONCENTRATIONS OF SOME HEAVY METALS IN SHELLFISH FROM 20 SAMPLING STATIONS IN HOWE SOUND, B.C. JUNE, 1981

SITE	(Zone No.)	ORGANISM	$ug \cdot g^{-1}$ wet weight					
			As	Cd	Pb	Zn	Cu	Hg
1) Gibsons Bay	(8)	Mya arenaria	1.78	0.74	1.74	14.3	4.51	< 0.039
2) Soame's Pt.	(8)	M. arenaria	2.34	.141	< .617	16.7	3.58	< 0.030
3) Grantham's Landing	(8)	Mytilus edulis	1.22	.594	< .582	34.4	1.81	0.030
4) YMCA Camp	(7)	M. edulis	1.25	.681	< .552	33.5	2.31	0.040
5) Langdale	(7)	M. arenaria	1.64	.093	< .541	11.8	5.76	< 0.028
6) YMCA Camp	(7)	M. arenaria	1.96	.168	< .59	14.6	4.78	< 0.029
7) Furry Creek	(4)	M. edulis	1.45	.709	< .48	42.5	4.31	0.030
8) Daisy Creek	(4)	M. edulis	1.51	.414	< .47	33.4	3.69	0.025
9) Porteau Cove	(5)	M. edulis	1.5	.591	< .553	23.1	3.03	0.033
10) Port Graves	(7)	Crassostrea gigas	1.17	1.39	< .552	601	39.4	0.033
11) Centre Bay	(7)	C. gigas	1.21	3.03	< .495	2060	249.0	0.034
12) West Bay	(7)	M. arenaria	1.95	.132	< .531	13.9	5.05	0.032
13) Potlatch Creek	(5)	M. edulis	1.11	.806	< .424	44.9	2.31	0.031
14) McNab Creek	(6)	M. edulis	1.09	.502	< .51	20.0	2.11	0.027
15) Douglas Bay	(6)	M. edulis	L.88	.458	< .468	27.5	2.38	< 0.026
16) Halkett Bay	(7)	M. arenaria	2.79	.088	< .549	13.1	5.42	0.027
17) Deep Bay 79	(8)	C. gigas	1.31	1.67	< .571	913	90.6	0.038
18) Deep Bay 81	(8)	M. edulis	1.08	.619	.482	33.8	3.76	0.032
19) Deep Bay 84	(8)	M. edulis	23.2	.639	< .636	15.2	6.86	0.037
20) Grafton Bay	(8)	M. arenaria	2.33	.103	< .714	13.1	5.68	< 0.035

APPENDIX II
TABLE 5

Zinc, copper and cadmium concentrations in oysters (Crassostrea gigas) near three B.C. pulp mills. See Figure 7 for distances of sites from pulp mills. Control is at Nanoose Bay, one of four used in the study (173, 174).

LOCATION	SITE NO.	$ug.g^{-1}$ dry wt. (wet wt.) Zn	Cu	Cd
Powell River	19 (near mill)	17,280 (2,120)	218 (26.8)	4.62 (0.57)
Powell River	23 (further east)	6,680 (856)	262 (33.8)	14.60* (2.0)
Crofton	D-10	10,020 (1,420)	220 (30.6)	10.20* (1.1)
Crofton	D-17 (nearest mill)	8,060 (1,150)	112 (16.8)	5.8 (0.8)
Campbell River	B-21**	5,360 (1,026)	222 (43.8)	10.86* (1.9)
Campbell River	B-18	4,660 (782)	144 (23.4)	12.86* (2.7)
Nanoose Bay	F-1	2,960 (545.2)	170 (27.4)	12.78* (2.1)

* - high values for cadmium and copper at stations removed from mills.
** - closest oyster population to Elk Falls mill was three miles distant.

APPENDIX II
TABLE 6 CROFTON OYSTER TISSUE ANALYSIS - TRACE METALS, JANUARY 1977 (177)

SAMPLE STATION	1977 DATE OF SAMPLING	REPLICATE	Mercury Hg (ug/g)		Zinc Zn (ug/g)		Cadmium Cd (ug/g)	
			wet	dry	wet	dry	wet	dry
0-1	Jan. 19	A	0.10	0.59	520	3100	1.6	9.4
		B	0.09	0.54	520	3100	1.4	8.5
0-2	Jan. 19	A	0.05	0.58	580	6200	1.1	11.0
		B	0.06	0.60	620	6300	1.1	11.0
0-3	Jan. 19	A	0.05	0.53	460	5200	0.8	9.1
		B	0.05	0.55	467	5200	0.8	9.2
0-4	Jan. 25	A	0.05	0.36	520	4100	0.8	6.0
		B	0.04	0.33	540	4200	0.8	6.0
0-5	Jan. 25	A	0.05	0.39	570	4300	0.7	5.0
		B	0.06	0.40	600	4300	0.7	5.0
0-7	Jan. 25	A	0.09	0.39	980	4150	1.0	6.0
		B	0.09	0.43	940	4300	1.0	6.0
0-8	Jan. 25	A	0.08	0.29	880	3200	2.0	6.0
		B	0.10	0.38	860	3200	2.0	7.0
0-9	Jan. 25	A	0.08	0.34	770	3500	2.0	7.0
		B	0.07	0.33	730	3300	2.0	7.0
0-10	Jan. 25	A	0.08	0.40	720	3600	1.0	7.0
		B	0.04	0.26	550	3700	1.0	7.0
0-2	Feb. 1	A	0.05	0.34	450	3100	1.2	8.4
		B	0.06	0.39	460	3100	1.3	8.9
0-5	Feb. 1	A	0.04	0.39	490	5400	0.58	6.4
		B	0.03	0.37	480	5300	0.52	6.0
0-8	Feb. 1	A	0.05	0.37	700	6000	1.1	8.4
		B	0.04	0.31	680	5900	1.0	8.6

Replicate samples not taken.

APPENDIX II

TABLE 7 MEAN ZINC (mg/g dry tissue) AND CONDITION FACTOR LEVELS AVERAGED BY SIZE CLASSES OF OYSTERS, CROFTON, B.C. (178)

Two-way ANOVAs, n = 15/cell, SD in parentheses

	Zinc		Condition Factor	
	Test	Control	Test	Control
Small	4.76 (2.03)	2.12 (0.87)	28.2 (8.8)	155.5 (33.6)
Medium	6.26 (1.85)	1.45 (0.80)	24.9 (10.6)	150.3 (21.4)
Large	5.85 (2.76)	1.96 (1.10)	24.4 (8.2)	148.1 (18.1)
$\bar{x}$	5.69	1.84	25.8	151.3

APPENDIX II

TABLE 8 COMPARISON OF MEAN TRACE METAL CONCENTRATIONS (mg/kg dry weight) IN *M. edulis* TISSUES IN ALICE ARM AND HASTINGS ARM (1977, 1978, 1980, 1981) (179)

SURVEY AND STATION		WET/DRY RATIO	Cu	Pb	Zn	Cd	As	Mo	Ni	Cr	Fe	Mn	Mg	Al	Hg
ALICE ARM															
AAN	1977	.00	8.9	2.60	110.0	4.80	0	.00	.0	.00	380.0	.00	0	0	0
AAN	1978	6.20	12.8	< 4.30	59.3	3.50	7	< 7.20	.0	1.91	1000.0	.00	0	0	0.191
LC	1977	.00	11.0	5.00	130.0	6.60	0	.00	.0	.00	450.0	.00	0	0	0
LC	1978	6.30	12.9	7.81	107.0	5.86	9	< 7.30	.0	1.95	1100.0	.00	0	0	0.109
LC (purged)	1978	8.10	22.5	4.84	102.0	6.82	10	< 7.20	.0	1.74	662.0	.00	0	0	0.113
Outfall B															
May-June	1981	11.82	11.2	4.30	125.3	9.46	12	.69	2.6	2.20	465.7	18.45	2491	202	0
A-1-N	1977	.00	9.6	2.10	100.0	6.50	0	.00	.0	.00	810.0	.00	0	0	0
A-1-N	1978	7.90	14.9	< 4.30	101.0	7.40	9	< 7.20	.0	2.94	2220.0	.00	0	0	0.137
A-1-S	1978	7.70	18.3	5.60	107.0	5.90	12	< 7.30	.0	2.23	1010.0	.00	0	0	0.114
A-2-N	1977	.00	13.0	2.10	110.0	6.90	0	.00	.0	.00	260.0	.00	0	0	0
A-2-N	1978	7.30	25.5	< 4.35	93.0	6.82	10	< 7.25	.0	3.05	1400.0	.00	0	0	0.161
A-2-S	1977	.00	10.0	1.10	99.0	6.00	0	.00	.0	.00	150.0	.00	0	0	0
A-2-S	1978	9.20	14.9	< 4.30	101.0	7.42	9	< 7.20	.0	2.94	2200.0	.00	0	0	< 0.098
A-3-N	1978	6.60	11.6	< 4.30	82.7	3.50	8	< 7.15	.0	1.61	789.5	.00	0	0	0
A-3-S	1978	6.20	12.0	< 4.30	34.0	3.44	7	< 7.20	.0	1.76	726.0	.00	0	0	< 0.088
HASTINGS ARM															
H-2-E	1977	.00	9.6	< 1.00	87.0	4.50	0	.00	.0	.00	260.0	.00	0	0	0
H-2-E May-June	1981	6.74	13.4	2.28	86.6	5.34	8	.32	1.4	1.30	364.4	17.85	2849	180	0
H-2-W	1977	.00	10.0	< 1.00	89.0	5.80	0	.00	.0	.00	440.0	.00	0	0	0
H-2-W May-June	1981	7.28	16.2	2.40	131.0	7.06	10	.44	1.9	1.68	694.5	24.85	2684	339	0
Dobin I.E.															
May-June	1981	8.38	15.2	2.30	162.3	7.50	11	.38	1.7	1.78	587.9	17.33	3393	275	0
H-3-E	1978	7.50	14.9	< 4.35	80.7	4.34	8	< 7.30	.0	1.31	837.0	.00	0	0	0
ANYOX															
H-4-N	1977	.00	27.0	< 1.00	230.0	3.30	0	.00	.0	.00	430.0	.00	0	0	0
H-4-N	1978	8.50	243.0	7.20	462.0	8.36	11	< 7.20	.0	2.98	5910.0	.00	0	0	0
B	1978	8.40	53.3	< 4.20	233.0	7.88	11	< 6.90	.0	2.73	1120.0	.00	0	0	0
GP	1978	7.30	120.0	< 4.30	202.0	5.60	8	< 7.20	.0	1.21	699.0	.00	0	0	0
L	1978	6.80	23.9	< 4.40	111.0	3.77	9	< 7.30	.0	2.33	1060.0	.00	0	0	0
JP	1978	7.70	128.0	< 4.20	264.0	7.46	8	< 7.00	.0	2.98	5910.0	.00	0	0	0

0 = not analyzed

APPENDIX II

TABLE 9 AVERAGE METAL LEVELS IN BIOTA, AMAX SITE (pre-operational 1974-77)
Values in mg/kg Dry Weight (ppm) (180)

ORGANISM	COPPER	ZINC	LEAD	CADMIUM	MOLYBDENUM
1. Rockweed	13.3	39.3	9.1	2.8	ND*
$S_{\bar{x}}$	1.56	4.03	1.54	0.11	--
N	15	15	15	16	15
2. Zooplankton	17.3	262.1	20.0	3.8	ND
$S_{\bar{x}}$	2.61	52.48	2.62	0.47	--
N	4	4	4	4	4
3. Cockles	27.7	173.0	20.0	3.8	ND
$S_{\bar{x}}$	17.6	40.28	0.77	0.14	--
N	4	4	4	4	4
4. Mussels	20.7	129.0	18.8	8.1	ND
$S_{\bar{x}}$	2.34	10.09	2.71	0.58	--
N	15	15	15	15	15
5. Shrimp	42.1	53.4	3.9	1.0	ND
$S_{\bar{x}}$	1.30	5.10	0.44	0.11	--
N	2	2	2	2	2
6. Spider crab	42.3	377.3	7.9	2.4	ND
$S_{\bar{x}}$	6.4	25.75	0.36	0.60	--
N	5	6	5	6	6
7. Brown King crab	147.9	216.0	1.8	0.3	1.2
$S_{\bar{x}}$	11.32	6.70	0.57	0.03	0.13
N	6	6	6	6	6
8. Tanner crab	50.5	212.3	0.8	0.8	ND
$S_{\bar{x}}$	5.06	23.07	0.09	0.15	--
N	6	6	6	6	6
9. Dungeness crab	64.6	247.0	3.5	1.0	1.4
$S_{\bar{x}}$	5.71	17.06	0.51	0.23	0.14
N	14	14	13	7	4

Continued...

APPENDIX II

TABLE 9 AVERAGE METAL LEVELS IN BIOTA, AMAX SITE (pre-operational 1974-77)
Values in mg/kg Dry Weight (ppm) (180)

(Continued)

ORGANISM	COPPER	ZINC	LEAD	CADMIUM	MOLYBDENUM
10. Yellowfin sole	23.3	92.4	13.8	10.9	ND
$S_{\bar{x}}$	2.08	7.61	2.42	2.05	--
N	15	16	16	16	16
11. Great sculpin	25.6	149.9	4.2	3.0	ND
$S_{\bar{x}}$	4.46	11.79	0.48	0.39	--
N	13	14	15	15	15
12. Crow	18.6	108.2	4.2	12.7	ND
$S_{\bar{x}}$	0.30	3.85	0.43	0.73	--
N	2	2	2	2	2
Mean value	42.22	168.3	7.94	4.04	--
$S_{\bar{x}}$	10.74	25.95	1.85	1.21	--
Maximum	147.9	377.3	20.0	12.7	1.4
Minimum	13.3	39.3	1.8	0.3	1.2

*Not Detectable

APPENDIX II

TABLE 10 METAL CONCENTRATIONS, ($ug.g^{-1}$) (ppm) IN BIVALVE MOLLUSCS OF RUPERT AND HOLBERG INLETS, 1971-1974 (182)

SPECIES	STATION	DATE	# OF SPECIMENS	Zn		Cu	
				Dry Wt.	Wet Wt.	Dry Wt.	Wet Wt.
Macoma irus	Red Is. (Rupert Inlet)	Aug./71	6	260	44	460	79
		June/72	6	140	18	100	14
		June/73	4	380	53	350	50
			4	300	46	310	48
	Scuba Station 6 (West of Mill Site)	June-Aug. 1971	3	320	43	59	8.0
			3	370	54	42	6.3
			11	110	24	71	15
		Sep./71	1	170	32	70	14
		June/73	3	230	28	130	16
			3	300	44	38	5.6
			3	280	39	53	8.2
	Apple Bay (Holberg Inlet)	June/73	6	180	27	9	1.4
		May/74	2	150	18	61	7.3
			2	180	21	17	2.0
			2	140	18	16	2.1
			2	160	23	19	2.8
			2	150	18	59	7.4
Mya arenaria	Red Is.	Aug./71	6	105	16	77	12
		June/72	4	68	9	34	4.4
		June/73	2	140	19	31	4.1
			3	130	17	33	4.1
			3	120	16	29	3.8
		May/74	2	83	12	27	3.8
			2	78	13	25	4.1
			2	74	10	33	4.6
			2	110	12	30	3.3
			2	61	11	24	4.1

Continued...

APPENDIX II

TABLE 10 METAL CONCENTRATIONS, ($ug.g^{-1}$) (ppm) IN BIVALVE MOLLUSCS OF RUPERT AND HOLBERG INLETS, 1971-1974 (182) (Continued)

SPECIES	STATION	DATE	# OF SPECIMENS	Zn		Cu	
				Dry Wt.	Wet Wt.	Dry Wt.	Wet Wt.
Mya arenaria	Scuba Station 6	Aug./71	3	160	26	13	3.0
			4	110	18	24	4.0
		Sep./71	2	80	12	31	4.5
			2	160	22	31	4.4
		June/73	1	180	19	30	3.2
			1	170	17	27	2.8
			2	150	16	37	3.8
		May/74	1	110	13	18	2.3
			1	42	7	25	4.4
			1	50	10	12	2.5
			1	65	12	31	5.5
			1	48	9	25	4.0
	Apple Bay	June/72	2	140	13	17	1.6
			6	140	11	16	1.4
		June/73	2	130	17	55	7.2
			3	160	17	13	1.4
			3	110	13	15	1.9
		May/74	1	69	9	16	2.1
			1	48	7	22	3.3
			1	62	9	29	4.3
			1	68	10	19	2.7
Saxidomus giganteus	Red Is.	June-Aug. 1971	1	115	15	55	7.1
			1	250	39	40	6.3
			1	270	35	30	3.9

Continued...

APPENDIX II

TABLE 10 METAL CONCENTRATIONS, ($ug.g^{-1}$) (ppm) IN BIVALVE MOLLUSCS OF RUPERT AND HOLBERG INLETS, 1971-1974 (182) (Continued)

SPECIES	STATION	DATE	# OF SPECIMENS	Zn		Cu	
				Dry Wt.	Wet Wt.	Dry Wt.	Wet Wt.
Saxidomus giganteus	Red Is.	June-Aug.	1	160	23	7	1.1
		1971	1	86	15	13	0.4
			1	90	16	40	7.2
		June/72	1	120	16	16	2.1
			1	81	8	25	2.3
		June/73	2	97	14	32	4.6
			2	110	16	29	3.9
			2	120	15	52	6.2
		May/74	1	69	13	14	2.6
			1	88	18	13	2.6
			1	54	12	11	2.2
			1	60	11	10	1.8
			1	56	8	16	2.2
	Scuba Station 6	June-Aug.	1	110	17	27	4.2
		1971	1	110	16	24	3.5
			1	120	15	34	4.3
			1	90	34	26	9.7
			2	82	17	17	3.7
		Sep./71	1	73	14	12	2.2
			1	56	10	17	2.9
		June/73	1	100	18	23	3.9
			1	120	22	19	3.6
			1	98	16	17	2.7

Continued...

APPENDIX II
TABLE 10 METAL CONCENTRATIONS, ($ug.g^{-1}$) (ppm) IN BIVALVE MOLLUSCS OF RUPERT AND HOLBERG INLETS, 1971-1974 (182) (Continued)

SPECIES	STATION	DATE	# OF SPECIMENS	Zn		Cu	
				Dry Wt.	Wet Wt.	Dry Wt.	Wet Wt.
Saxidomus giganteus	Scuba Station 6	May/74	1	100	21	23	4.7
			1	100	25	19	4.6
			1	69	17	13	3.2
			1	49	11	18	4.0
			1	85	19	20	4.5
	Apple Bay	June/73	1	120	20	17	2.8
			1	96	13	7	1.3
			1	100	16	11	1.7
		May/74	1	55	12	9	2.0
			1	86	18	10	2.1
			1	56	11	14	2.8
			1	53	11	11	2.2
			1	54	10	10	1.8
Protothaca staminea	Red Is.	Aug./71	6	92	15	10	1.7
			12	110	17	13	2.0
		June/72	2	110	12	20	2.1
		June/73	5	150	19	37	4.7
			5	120	15	37	4.6
			5	140	17	45	5.5
		May/74	1	57	11	18	3.3
			1	70	13	19	3.5
			1	110	20	15	2.7
			1	92	15	22	3.6
	Scuba Station 6	Aug./71	7	110	18	15	2.4
			7	87	17	14	2.7

Continued...

APPENDIX II
TABLE 10 METAL CONCENTRATIONS, ($ug.g^{-1}$) (ppm) IN BIVALVE MOLLUSCS OF RUPERT AND HOLBERG INLETS, 1971-1974 (182) (Continued)

SPECIES	STATION	DATE	# OF SPECIMENS	Zn		Cu	
				Dry Wt.	Wet Wt.	Dry Wt.	Wet Wt.
Protothaca staminea	Scuba Station 6	Sep./71	7	78	12	26	3.8
		June/73	2	12	2	30	3.8
			2	150	15	31	3.1
			3	120	17	40	5.4
		May/74	1	67	14	12	2.6
			1	86	16	15	2.9
			1	120	22	16	3.1
			1	69	12	13	2.4
	Apple Bay	June/73	1	62	11	15	2.6
			5	110	13	19	2.2
			5	140	17	14	1.8
		May/74	5	130	16	13	1.6
			1	97	7	24	4.4
			1	110	20	13	2.4
			1	99	16	11	1.8
			1	83	13	14	2.2
			1	88	12	15	2.2
Mytilus edulis	Red Is.	July-Aug. 1971	18	340	14	44	7.8
			25	89	16	16	6.2
		June/73	7	220	25	87	10
		May/74	3	33	13	20	2.8
			3	120	16	13	1.8
			3	120	15	14	17
			3	120	-	14	-

Continued...

APPENDIX II

TABLE 10 METAL CONCENTRATIONS, ($ug.g^{-1}$) (ppm) IN BIVALVE MOLLUSCS OF RUPERT AND HOLBERG INLETS, 1971-1974 (182) (Continued)

SPECIES	STATION	DATE	# OF SPECIMENS	Zn		Cu	
				Dry Wt.	Wet Wt.	Dry Wt.	Wet Wt.
Mytilus edulis	Scuba Station 6	July-Aug. 1971	12	190	30	52	8.4
			26	100	19	17	3.3
		June/73	9	190	17	43	4.0
		May/74	2	63	84	16	2.1
			2	110	16	17	2.4
			2	87	12	12	1.6
			2	130	19	10	1.4
			2	75	13	13	2.2
	Apple Bay	June/72	2	51	12	5	1.2
		June/73	6	120	18	9	1.4
		May/74	2	51	12	5	1.2
			2	62	11	12	2.3
			2	64	11	7	1.1
			2	95	12	10	1.3
			2	83	13	13	2.0

APPENDIX II

TABLE 11 STATISTICAL COMPARISON OF COPPER, ZINC, AND ARSENIC LEVELS IN SHELLFISH COLLECTED AT THE ISLAND COPPER MINE SITE (183)

TIME PERIOD	METAL (ppm)	H 1 Mean ± S.D.	H 2 Mean ± S.D.	R 4 Mean ± S.D.	R 6 Mean ± S.D.	Q 7 Mean ± S.D.	ANOVA Sign. Level
Protothaca staminea							
71/06	Cu	1.46 ± 0.55	1.21 ± 0.40	1.81 ± 0.85	2.32 ± 0.80	0.94 ± 0.45	P 0.05
72/09		(13)	(12)	(9)	(2)	(13)	
	Zn	11.0 ± 3.8	10.2 ± 2.1	10.3 ± 1.8	11.3 ± 0.1	8.3 ± 1.4	N.S.
		(13)	(12)	(9)	(2)	(13)	
	As	1.76 ± 0.9	1.23 ± 0.76	1.04 ± 0.65	-	2.08 ± 0.9	N.S.
		(8)	(8)	(12)		(11)	
76/12	Cu	1.01 ± 0.38	1.01 ± 0.21	1.87 ± 0.49	0.99 ± 0.30	0.97 ± 0.27	P 0.025
78/09		(9)	(7)	(6)	(6)	(9)	
	Zn	14.5 ± 2.3	14.8 ± 2.4	16.9 ± 4.4	10.4 ± 3.2	10.4 ± 3.0	P 0.001
		(9)	(7)	(6)	(6)	(6)	
	As	1.17 ± 0.43	1.71 ± 0.49	1.49 ± 0.17	2.27 ± 1.0	2.84 ± 1.0	P 0.0005
		(9)	(7)	(6)	(6)	(6)	
Mya arenaria							
71/06	Cu	1.21 ± 0.50	0.73 ± 0.25	1.32 ± 0.32	1.36 ± 0.46	1.11 ± 0.54	N.S.
72/09		(15)	(6)	(11)	(12)	(11)	
	Zn	8.3 ± 1.5	9.0 ± 2.6	8.7 ± 1.8	8.0 ± 1.6	9.0 ± 2.8	N.S.
		(15)	(6)	(11)	(12)	(11)	
	As	0.77 ± 0.23	0.84 ± 0.22	0.87 ± 0.16	0.49 ± 0.17	0.78 ± 0.38	N.S.
		(15)	(6)	(11)	(12)	(11)	
76/12	Cu	1.53 ± 0.52	1.67 ± 0.41	2.43 ± 0.65	1.31 ± 0.44	1.71 ± 0.55	P 0.025
78/09		(9)	(9)	(9)	(9)	(9)	
	Zn	9.6 ± 1.5	9.8 ± 1.6	10.5 ± 2.3	9.7 ± 2.9	8.9 ± 1.7	N.S.
		(9)	(9)	(9)	(9)	(9)	
	As	1.1 ± 0.22	1.1 ± 0.40	1.2 ± 0.3	0.9 ± 0.3	1.0 ± 0.3	N.S.
		(9)	(9)	(9)	(9)	(9)	

Continued...

APPENDIX II

TABLE 11 STATISTICAL COMPARISON OF COPPER, ZINC, AND ARSENIC LEVELS IN SHELLFISH COLLECTED AT THE ISLAND COPPER MINE SITE

(Continued)

TIME PERIOD	METAL (ppm)	Coal Hbr. Dock Mean ± S.D.	Quatsino Dock Mean ± S.D.	Utah Dock Mean ± S.D.	ANOVA Sign. Level
Mytilus edulis					
74/03	Cu	1.13 ± 0.53	1.18 ± 0.52	2.08 ± 0.19	P 0.0005
75/06		(18)	(18)	(38)	
	Zn	11.3 ± 3.0	11.4 ± 5.0	13.3 ± 5.1	N.S.
		(18)	(18)	(38)	
	As	1.08 ± 0.37	1.02 ± 0.51	1.19 ± 0.37	N.S.
		(18)	(18)	(38)	
75/06	Cu	1.29 ± 0.20	1.07 ± 0.09	1.60 ± 0.38	P 0.0005
78/09		(24)	(24)	(24)	
	Zn	17.0 ± 7.4	9.8 ± 3.4	17.6 ± 6.5	P 0.0005
		(24)	(24)	(24)	
	As	1.0 ± 0.4	0.90 ± 0.46	0.90 ± 0.39	N.S.
		(24)	(24)	(24)	

Note: The number in brackets denotes sample size. Metal levels expressed in ppm wet weight. (H) denotes a Holberg Inlet station, (Q) Quatsino Sound station, and (R) a Rupert Inlet station. The station locations are indicated in Figure 11.

APPENDIX II

TABLE 12 MEAN (± S.D.) TISSUE COPPER, ZINC, AND ARSENIC LEVELS IN *H. kennerleyi* [(ppm) - wet weight] (183)

MONTH	n	COPPER Mean ± S.D.	ZINC Mean ± S.D.	ARSENIC Mean ± S.D.
Apr. '76	6	0.75 ± 0.12	6.6 ± 1.8	5.0 ± 2.4
June '76	6	1.1 ± 0.50	7.8 ± 1.3	2.5 ± 1.5
Sept. '76	3	0.64 ± 0.23	9.4 ± 2.2	2.3 ± 0.6
June '77	3	1.1 ± 0.2	10.0 ± 2.2	3.3 ± 1.7
Aug. '77	3	-	11.0 ± 1.9	5.5 ± 1.9
Jan. '78	6	0.61 ± 0.15	9.8 ± 2.0	3.4 ± 2.1
Apr. '78	4	0.63 ± 0.14	6.9 ± 0.45	3.8 ± 0.8
June '78	3	0.84 ± 0.18	8.3 ± 2.4	2.0 ± 0.9
Sept. '78	3	0.80 ± 0.40	6.9 ± 1.2	3.8 ± 1.5

APPENDIX II
TABLE 13 ISLAND COPPER MINE METAL ANALYSIS DATA (1975-1980) (183)

Mytilus edulis - Utah Dock

n = 3 unless otherwise noted
* - n = 4; ** - n = 2

DATE	Pb	Cd	Hg	As	Cu	Zn	Mo
1975							
March	0.55	0.28	0.047	1.67	2.40	39.03	0.24
June	L0.10	0.31	0.009	0.77	1.49	15.42	0.12
Sept.	L0.10	0.34	0.018	0.98	2.43	17.55	0.36
Dec.	0.43	0.28	0.017	0.64	1.63	20.38	0.27
1976							
March	0.23	0.26	0.014**	0.78	2.19	14.94	0.16
June	0.42	0.28	0.020	1.4	2.16	15.35	0.30
Sept.							
Dec.	0.11	0.30	0.011	0.5	0.75	18.71	< 0.10
1977							
March	L0.10	0.35	0.016	2.8	2.83	14.38	0.35
June	0.92	0.52	0.02	0.9	2.03	43.03	< 0.20
1978							
March	1.1	0.46	0.007	1.4	3.5	27.5	0.3
June	0.9	0.35	0.007	1.3	2.7	20.3	0.1
Sept.	0.7	0.27	0.007	0.7	2.1	10.0	0.2
Dec.	0.7	0.28	0.02	0.6	1.1	11.0	0.1
1979							
March	0.9	0.27	0.02	1.1	4.7	16.0	< 0.1
June	1.6	0.58	0.01	1.4	2.3	22.0	< 0.1
Sept.	0.5	0.46	0.02	1.7	1.5	29.0	< 0.1
Dec.	1.2	0.52	0.01	0.9	2.1	47.0	< 0.2
1980							
March	0.7	0.55	0.02	1.3	1.7	41.0	0.1

Continued...

APPENDIX II
TABLE 13 ISLAND COPPER MINE METAL ANALYSIS DATA (1975-1980) (183)
(Continued)

Mytilus edulis - Coal Harbour Dock

n = 3 unless otherwise noted
* - n = 4; ** - n = 2

DATE	Pb	Cd	Hg	As	Cu	Zn	Mo
1975							
March	0.34	0.24	0.028	0.88	1.30	9.51	0.19
June	< 0.10	0.30	0.008	1.20	1.51	17.15	< 0.05
Sept.	< 0.10	0.33	0.010	0.98	1.05	21.02	< 0.10
Dec.	0.28	0.27	0.015	0.73	0.95	18.43	0.16
1976							
March	0.21	0.48	0.029	0.94	1.70	24.55	0.12
June	0.27	0.63	0.020	1.0	1.15	25.40	< 0.10
Sept.							
Dec.	0.70	0.44	0.068	1.1	2.76	14.40	< 0.10
1977							
March	< 0.10	0.31	0.055	2.4	1.69	23.17	0.23
June	0.33	0.43	0.042	0.7	1.15	14.47	< 0.10
1978							
March	1.2	0.54	0.106	1.7	1.7	19.1	0.1
June	0.4	0.40	0.013	1.3	1.5	19.7	0.1
Sept.	0.6*	0.43*	0.007**	0.9*	1.3*	15.6*	0.2*
Dec.	0.6	0.29	0.08	0.7	0.9	12.0	0.1
1979							
March	0.9	0.34	0.03	1.4	2.0	16.6	< 0.1
June	0.7	0.36	0.02	1.5	1.9	24.0	0.2
Sept.	0.2	0.29	0.05	1.4	1.1	21.0	< 0.2
Dec.	0.3	0.42	0.04	2.0	1.7	30.0	0.1
1980							
March	0.1	0.28	0.02	1.0	1.1	24.0	< 0.1

Continued...

APPENDIX II
TABLE 13 ISLAND COPPER MINE METAL ANALYSIS DATA (1975-1980) (183)

(Continued)

Mytilus edulis - Quatsino Dock

n = 3 unless otherwise noted

DATE	Pb	Cd	Hg	As	Cu	Zn	Mo
1975							
March	0.36	0.41	0.012	0.83	1.03	9.77	0.24
June	< 0.10	0.31	0.017	0.77	1.12	15.42	0.05
Sept.	< 0.10	0.62	0.017	0.78	1.03	11.46	< 0.10
Dec.	0.52	0.51	0.017	0.61	1.06	9.62	< 0.10
1976							
March	0.30	0.39	0.022	0.73	1.52	9.66	0.12
June	0.22	0.33	0.021	0.50	1.01	6.69	0.05
Sept.							
Dec.	0.24	0.58	0.023	0.60	0.70	10.72	0.15
1977							
March	1.04	0.31	0.017	2.8	1.20	11.1	< 0.10
June	0.82	0.91	0.028	0.7	1.42	19.8	< 0.23
1978							
March	1.4	1.30	0.02	1.5	1.9	10.1	< 0.1
June	0.2	0.63	0.004	1.1	1.3	12.9	0.1
Sept.	0.5	1.26	0.005	0.9	1.3	10.2	0.1
Dec.	0.7	0.28	0.01	2.4	0.9	20.0	0.4
1979							
March	0.6	0.36	0.01	0.8	1.0	6.8	< 0.1
June	1.1	0.4	0.01	1.3	1.7	15.0	< 0.1
Sept.	0.3	0.8	0.03	1.6	1.2	20.3	0.1
Dec.	0.5	0.42	0.02	2.0	1.3	21.0	0.2
1980							
March	0.9	0.75	0.04	1.6	1.1	7.8	< 0.1

APPENDIX III

LEVELS OF CARCINOGEN BENZO(a)PYRENE IN COMMERCIAL SHELLFISH FROM B.C.

APPENDIX III: LEVELS OF CARCINOGEN BENZO(a)PYRENE IN COMMERCIAL SHELLFISH FROM B.C.

Samples were obtained June 8, 1979 from Wayne Holmes, Vancouver Island Fish Inspection Supervisor, and had been collected in the spring of 1979. For comparison with data from other samples, compare the listed values for benzo(a)pyrene with the values for benzo(a)pyrene in commercial seafoods in "Polycyclic Aromatic Hydrocarbons in Commercial Seafoods" by B.P. Dunn and J. Fee in J. Fisheries Research Board Canada, 36, 1469-1476 (1979) (139).

Locations

1. Oysters from Henry Bay
2. Oysters from Denman Island
3. Oysters from Comox Harbour
4. Oysters from Cortes Island
5. Oysters from Barkley Sound
6. Butterclams from Seal Islets
7. Geoducks from Courtenay area

ANALYTICAL RESULTS ng/g WET WEIGHT

COMPOUND	CARCINO-GENICITY	Location 1	2	3	4	5	6	7
Fluoranthene	-	-*	31	23.5	96.5	-	6.7	44
Benz(a)anthracene	+	5.0	4.1	3.8	1.8	-	1.7	3.9
Benzo(b)fluoranthene	++	5.1	4.9	3.8	3.8	2.0	0.6	3.2
Benzo(k)fluoranthene	-	1.8	1.9	1.2	1.7	0.6	0.1	0.9
Benzo(a)pyrene	+++	0.5	0.8	0.5	0.5	0.3	0.4	0.9
Indeno(1,2,3-cd)-pyrene	+	0.4	0.5	0.3	0.5	0.2	0.3	0.6
Benzo(b)chrysene	?	0.3	0.3	0.4	0.2	0.1	0.5	0.4

*Indicates analysis impossible because of technical limitations.

APPENDIX IV

PCB CONCENTRATIONS IN BIVALVE MOLLUSCS OF BRITISH COLUMBIA (1977-1981)

APPENDIX IV PCB CONCENTRATIONS IN BIVALVE MOLLUSCS OF BRITISH COLUMBIA 1977-1981 ($ug.kg^{-1}$ wet weight) Adapted from Garrett (168)

LOCATION	SPECIES	DATE	NO. OF SAMPLES	PCB CONCENTRATION Mean ± S.D.	Range
Fraser River Estuary					
Sturgeon Bank	clams	Aug/78	composite	ND	
		Nov/78	composite	0.05*	
	clams	Aug/78	composite	ND	
		Nov/78	composite	0.3	
Roberts Bank	clams	Aug/78	composite	ND	
		Nov/78	composite	76.0	
		1980	composite	< 20.0	
False Creek under N. Arm Burrard Bridge	mussels	Aug/78	composite	14.0	
under N. end Granville St. Bridge	mussels	Aug/78	composite	17.0	
Burrard Yarrows Shipyard, Burrard Inlet	mussels	1980	composite	400.0	
Bowyer Island, Howe Sound	mussels	1980	composite	20.0	

*Concentrations below level of confident quantification.

Continued...

APPENDIX IV PCB CONCENTRATIONS IN BIVALVE MOLLUSCS OF BRITISH COLUMBIA 1977-1981 ($ug.kg^{-1}$ wet weight) Adapted from Garrett (168)

LOCATION	SPECIES	DATE	NO. OF SAMPLES	PCB CONCENTRATION Mean ± S.D.	Range
Prince Rupert area					
Porpoise Harbour					
Stn. F immediately adjacent spill area	mussels	Jun/77	composite	17,000	
Stn. D - S. of Kaien Isl.	mussels	Jun/77	composite	254.0	
Stn. B. - off Ridley Isl.	clams	Jun/77	composite	87.0	
E. coast	mussels	Jun/77	composite	216.0	
Stn. E. - off B.C. Packers	mussels	Jun/77	composite	179.0	
Stn. H - W. (3.3 mi. W. of Port Edward (control stn.))	mussels	Jun/77	composite	9.0	
Alice Arm area	mussels	Oct/80	composite	100.0	
Lime Creek	mussels	Feb/81	1	1700.0	
	mussels	May/81	composite (5)	86 ± 181	< 5-410
Roundy Creek	mussels	Oct/80	composite	210.0	
	cockle	Oct/80	1	< 20.0	
	mussels	Feb/81	composite 3	860.0 ± 140.0	720.0-1000.0
Perry Bay	mussels	Feb/81	composite 3	120.0 ± 20.0	110.0-140.0

Continued...

APPENDIX IV PCB CONCENTRATIONS IN BIVALVE MOLLUSCS OF BRITISH COLUMBIA 1977-1981 ($ug.kg^{-1}$ wet weight) Adapted from Garrett (168)

LOCATION	SPECIES	DATE	NO. OF SAMPLES	PCB CONCENTRATION Mean ± S.D.	Range
Alice Arm area near Kitsault Mine	mussels	May/81	composite (2)	40 ± 47	6 - 73
Nanoose Bay	oysters	Mar/77	composite	ND	
Piper's Lagoon	oysters	Mar/77	composite	ND	
Shoregrove Resort	oysters	Mar/77	composite	ND	
Crofton	oysters	Jan/77	7	ND	ND-ND

APPENDIX V

LEVELS OF VARIOUS TOXIC CONTAMINANTS IN SHELLFISH IN THE VICINITY OF MUNICIPAL OUTFALLS

APPENDIX V

TABLE 1 TRACE METAL LEVELS IN MUSSEL TISSUE - McMicking Point (188)

(Cu,Cd,Pb,Zn)

Concentrations of Trace Metals in Mussel Tissues (Cu,Cd,Pb,Zn)

STATION IDENTIFICATION	Concentration $ug.g^{-1}$ dry weight			
	COPPER	CADMIUM	LEAD	ZINC
M1 (W. of Outfall)				
(M. californianus)	9.4	1.6	4.3	153
M3 (control)				
(M. californianus)	7.9	3.5	ND	176
M3 (control)				
(M. edulis)	10.3	3.3	ND	221

ND = below detection limit (detection limit for lead is 1.1 ppm)

*triplicate analyses

APPENDIX V

TABLE 2 MERCURY CONCENTRATIONS IN MUSSEL TISSUES - McMicking Point (188)

Concentrations of Mercury in Mussel Tissues

STATION IDENTIFICATION	Concentration $ng.g^{-1}$ dry weight		
	a	b	average
M1 (W. of Outfall)			
(M. californianus)	209	192	201
M3 (control)			
(M. californianus)	87	87	87
M3 (control)			
(M. edulis)	163	147	155

APPENDIX V

TABLE 3 CONCENTRATIONS OF SELECTED ORGANIC CONTAMINANTS DETECTED IN MUSSEL TISSUE (*M. californianus*) - McMicking Point (188)

Concentration (ppb)

CONTAMINANT	M1 (W. of Outfall) Dry Wt.	M1 (W. of Outfall) Wet Wt.	M3 Control Dry Wt.	M3 Control Wet Wt.
Diethyl phthalate	ND	ND	6	0.8
Di-n-butyl phthalate	49	9.1	ND	ND
Butyl benzyl phthalate	ND	ND	ND	ND
Bis (2-ethylhexyl) phthalate	1270	234	ND	ND
Hexachlorobenzene (HCB)	1.8	0.33	1.8	0.24
Lindane	1.3	0.24	ND	ND
DDE	18	3.2	12	1.6
Methoxychlor	59	11	ND	ND
Aroclor 1242	tr	tr	ND	ND
Aroclor 1254	84	16	32	4.3

ND = not detected

tr = trace amount detectable

APPENDIX V

TABLE 4 TRACE METAL CONCENTRATIONS IN OYSTER TISSUE - Comox Harbour Area (189)

LOCATION	Concentrations ($ug.g^{-1}$ dry weight) Cu	Cd	Pb	Zn	Concentration ($ug.g^{-1}$ wet weight) Hg
Goose spit	27.8	2.73	0.66	558	0.04 (mean of
	25.7	2.64	1.04	557	12 samples)
Point Holmes	45.0	2.74	0.71	862	< 0.09
	48.4	2.70	0.88	838	
Cape Lazo	36.3	2.42	0.78	905	
	27.7	2.95	0.88	563	

APPENDIX VI

CONTAMINANT LEVELS IN BIVALVE MOLLUSCS OF THE FRASER RIVER ESTUARY

APPENDIX VI

TABLE 1 METAL LEVELS IN BIVALVES FROM STURGEON AND ROBERTS BANKS (ug/g Dry Wt.)(192)

Species and Collection Location	Cadmium Mean Range	Chromium Mean Range	Cobalt Mean Range	Copper Mean Range	Iron Mean Range	Lead Mean Range	Manganese Mean Range	Nickel Mean Range	Silver Mean Range	Zinc Mean Range	Mercury Mean Range
Crassostrea gigas	19.3	< 6	< 2	410	793	< 4.0	40.8	< 2	7.3	3,166	-
North Side Iona Island Jetty	17.9-20.1	< 6	< 2	355-470	448-1,136	< 4.0	35.7-43.6	< 2	5.4-9.1	2,909-3,622	-
Crassostrea gigas	17.7	< 6.9	< 2	477	1,617	6.3	36.4	< 2	8.1	3,294	-
South Side Iona Island Jetty	10.0-26.7	< 6-9.5	< 2	280-557	998-3,481	< 4.0-12.3	24.3-44.0	< 2	4.3-11.9	2,836-3,838	-
Crassostrea gigas	12.0	-	< 8	400	-	< 2.0	48.0	< 7	28.0	2,400	0.220
West End, North Side Iona Island Jetty											
Cardium corbis	< 1.0	-	< 6	9.4	-	< 2.0	13.0	9	< 1.4	120	0.080
Roberts Bank											
Mya arenaria	< 1.0	-	< 8	30.0	-	2.0	25.0	8	17.0	72.0	0.370
Sturgeon Bank, North of Iona Jetty											

APPENDIX VI
TABLE 2 METAL CONCENTRATIONS IN SOFT TISSUES OF CLAMS (Macoma, Cryptomya, Mya) FROM STURGEON AND ROBERTS BANKS (192)

METAL	AREA	ug/g Dry Weight				ug/g Wet Weight			
		SAMPLE SIZE	NO. OF INDIV.	MEAN CONCEN.	S.D.	SAMPLE SIZE	NO. OF INDIV.	MEAN CONCEN.	S.D.
Arsenic	A	8	463	8.12	1.67	8	463	1.26	0.28
	B	8	431	8.37	1.48	8	431	1.40	0.25
	C	7	339	7.36	1.29	7	339	1.18	0.26
	D	9	335	30.7*	66.1	9	335	4.93*	10.87
	D[1]	8	293	8.64	1.84	8	293	1.31	0.42
Cadmium	A	8	463	3.34	3.08	8	463	0.522	0.513
	B	8	431	10.6	23.5	8	431	1.50	3.14
	C	7	339	31.0*	62.7	7	339	4.10*	7.88
	C[1]	6	319	2.35	1.83	7	319	0.397	0.286
	D	9	335	3.24	3.79	9	335	0.524	0.642
Chromium	A	8	463	2.00	0.489	8	463	0.324	0.132
	B	8	431	2.37	0.937	8	431	0.399	0.166
	C	7	339	2.05	0.618	7	339	0.340	0.124
	D	9	335	1.52	0.258	9	335	0.228	0.048
Copper	A	8	463	39.1	30.4	8	463	6.21	4.65
	B	8	431	50.1	41.3	8	431	10.4	8.05
	C	7	339	23.8	11.2	7	339	3.85	1.88
	D	9	335	23.9	8.73	9	335	3.58	1.36
Iron	A	8	463	745	362	8	463	116	51.6
	B	8	431	610	369	8	431	103	61.6
	C	7	339	789	339	7	339	125	47.9
	D	9	335	927	404	9	335	135	43.9
Nickel	A	8	463	3.91	0.71	8	463	0.60	0.09
	B	8	431	5.36	1.38	8	431	0.89	0.23
	C	7	339	5.16	2.09	7	339	0.82	0.28
	D	9	335	5.22	2.14	9	335	0.76	0.20
Lead	A	8	463	4.17	0.27	8	463	0.65	0.13
	B	8	431	5.24	1.09	8	431	0.88	0.19
	C	7	339	4.27	0.62	7	339	0.69	0.14
	D	9	335	4.62	1.24	9	335	0.69	0.19
Zinc	A	8	463	304	91.6	8	463	48.6	17.8
	B	8	431	231	106	8	431	38.9	18.2
	C	7	339	229	73.9	7	339	37.2	12.9
	D	9	335	203	80.2	9	335	31.6	14.7

A - North of Iona Jetty.
B - South of jetty in the zone of influence.
C - South of Sturgeon Bank.
D - Roberts Bank.
S.D.- Standard deviation.
* - Dubious data.
1 - Means and standard deviation omitting questionable data.

APPENDIX VI

TABLE 3 MERCURY LEVELS IN FRASER ESTUARY *MACOMA INCONSPICUS*
(ppm. wet weight)

STATION		LOWER LIMIT	MEAN	UPPER LIMIT	STAND. DEV.	STAND. ERROR
1	.03	0.020	0.040	0.060	0.017	0.009
2	-	0.076	0.102	0.128	0.023	0.011
3	.05	0.084	0.172	0.260	0.076	0.038
4	.06	0.043	0.064	0.085	0.018	0.009
5	-	0.028	0.038	0.048	0.008	0.004
6	-	0.050	0.074	0.098	0.021	0.010
7	.03	0.049	0.074	0.099	0.022	0.011
8	.07	0.052	0.088	0.124	0.031	0.016
9	.04	0.029	0.042	0.055	0.011	0.005
12	-	0.062	0.066	0.070	0.005	0.002
13	-	0.067	0.092	0.117	0.022	0.011
14	-	0.100	0.138	0.176	0.033	0.017
15	.04	0.026	0.060	0.094	0.030	0.015
17	-	0.032	0.054	0.076	0.019	0.010
18	.03	0.039	0.050	0.061	0.010	0.005
19	.03	0.029	0.046	0.063	0.015	0.008
20	-	0.087	0.138	0.189	0.044	0.022
21	-	0.027	0.044	0.061	0.015	0.008
22	-	0.073	0.120	0.167	0.041	0.021
23	-	0.017	0.038	0.059	0.018	0.009
24	.03	0.039	0.050	0.061	0.010	0.005
25	.11	0.063	0.132	0.200	0.069	0.031
26	.02	0.033	0.054	0.075	0.018	0.069
27	.02	0.030	0.054	0.078	0.021	0.010
28	.03	0.022	0.040	0.058	0.016	0.008
29	-	0.033	0.050	0.067	0.017	0.007
30	-	-0.008	0.110	0.228	0.183	0.051
31	-	0.026	0.036	0.046	0.009	0.004
32	.02	0.066	0.100	0.134	0.030	0.015
33	-	0.074	0.118	0.162	0.038	0.019
34	.09	0.069	0.080	0.091	0.010	0.005
35	.05	0.021	0.027	0.033	0.009	0.003

Note: Stations sampled June, 1976 by Don DeMill, Environmental Protection Service data from unpublished manuscript.

APPENDIX VII

METHODS

APPENDIX VII

METHODS

All monitoring data have to be compared, often first in time and frequently in space. A recent ICES (International Council for Exploration of the Sea) Reports on Further Intercalibration Analyses in ICES Pollution Monitoring and Baseline Studies (195) involved more than forty laboratories in analyses of heavy metals and organochlorines. An editorial in Marine Pollution Bulletin stated that for large changes (greater than 100%) most of the available data are sufficient; but if there is need to determine small changes in levels of pollutant (less than 100%) the quality of much of the data is not acceptable. A stated example was that only two labs reported on toxaphene, which "may be the most abundant of the anthropogenic chlorinated hydrocarbons in the environment today". (195)

Methods used in sampling and analyzing bivalves and other organisms as indicators of toxic chemical marine pollutants vary from place to place and laboratory to laboratory. Efforts have been made on a broad scale to intercalibrate analytical methods and to standardize field procedures to help assure reliable and comparable interpretation of the data (195). While some of the research projects referred to herein have been of a finite nature, others have been part of continuing programmes, particularly those related to environmental monitoring. A compilation of field and laboratory procedures used in the latter type of studies is outlined below.

1.0 FIELD

The design of field sampling programmes depends upon the purpose and scope of the investigation. Many physical and chemical environmental factors are routinely monitored, e.g. temperature,

salinity, conductivity, dissolved oxygen, etc., in the seawater, whether the programme involves sampling and analysis of water, sediment, biota or all three. This outline includes only information directly related to biota sampling and analyses.

2.0 ORGANISMS

Once an area had been designated for monitoring purposes of any individual or group of contaminants, there was a tendency initially, to select organisms, randomly or otherwise, which happened to be present in some quantity (163). In recent years there has been a trend to find commercially used suitable bivalve organisms which are known to bioaccumulate toxic substances. These include species of mussels (genus Mytilus) and oysters (genera Ostrea and Crassostrea), and to a lesser degree clams. The clam genera Mya and Mercenaria have been used as pollution indicators on the east coast of North America (91, 99). The burrowing clam Macoma balthica has been used in B.C. as an indicator of sediment pollution (125, 126).

While analysis of organisms inhabiting a given site, related to a pollution source, may determine the presence or absence of a pollutant(s), such organisms cannot be used for time-integrated data. For most on-going monitoring, the practice of transplanting organisms seems essential as discussed in Section 2.1.4. This practice has been adopted in the "Mussel Watch" and other similar programmes. Occasionally the oyster Crassostrea gigas has been used in specific projects (84, 85, 96). Several workers have transplanted hatchery-reared oysters (84, 85, 96). The use of hatchery-reared animals helps to some extent to overcome natural in-population variability discussed below in section 3.0. Macoma balthica specimens were transplanted in cages to test for pollutants in the Iona sewage plant outfall (125, 126), but the practice has not been used to any great extent in monitoring chemical pollution in B.C. coastal waters.

3.0 NUMBERS

Numbers are crucial at each stage of a monitoring programme. The number of sampling sites is related to the extent of the project, the pollutants under consideration and the source. To indicate a point source might require numerous stations, 1 metre apart; or fewer stations 1 km apart might be sufficient to determine the extent of pollution (68). Too few stations can result in unreliable, and therefore unusable data; too many can result in confusing, redundant data and needless increased costs, as well as time to completion.

A minimal number of organisms must be sampled at each station to overcome inherent variability in animal/pollutant relationships. Such variability in the data can frustrate attempts to identify differing concentrations of a pollutant. The importance of the choice of sample size was most clearly demonstrated by Boyden and Phillips (96). Even after all the variables related to seasonality, age, size, sex and position in the water column had been taken into account, they found intraspecific variation affected appropriate sample size on a species basis (96). In a study of metal pollution in three populations of hatchery-reared oysters, they tested the sub-sample size on measurement of zinc concentrations. Keeping in mind that some residual variation is always encountered regardless of sample size, they found sudden increases in variability at a given point, characteristic of each population. For one population of Ostrea edulis the increase occurred between subsample sizes of 15 and 10; in a second population of O. edulis the increase was between 20 and 15; in the third, a population of Crassostrea gigas the change occurred between 25 and 20 specimens. They concluded that the minimum number of individuals required to give accurate zinc concentrations in these three populations was 15, 20 and 25 respectively (96). In an earlier study Phillips found that concentrations of copper in Mytilus edulis were too variable for this organism to be used for indication of copper pollution (61, 62, 75).

Numbers also enter into the appropriate times of sampling, which in turn are related to the specific project. Choice of sampling times can incorporate or avoid seasonal changes either in the life cycle of the organism or in the appearance of the pollutant. For example, Dunn (135) attributed changes in benzo(a)pyrene concentrations to seasonal changes in effluent discharge rather than to changes in the organism.

4.0 OTHER

Transplants - Several methods have been used for transplanting suitable bivalves from one location to another to monitor pollution. These include trays (oysters) (84) and, cages (clams) (125) for placing organisms on the sediment. In the California Mussel Watch, the animals have been enclosed in mesh bags and attached to piers, floats or dowels stuck in the sediment, allowing for tidal changes to be included or not, as desired (63, 65).

The handling of animals between the field station and the laboratory is of obvious importance. Every precaution needs to be taken to assure that extraneous contamination of the organisms does not occur, for example from petroleum products in a boat.

5.0 LABORATORY ANALYSES

Laboratory methods refer to bioassays and chemical analyses for heavy metals and organochemicals. Techniques to assess chemical pollutants in the marine environment have been adapted from those used to monitor freshwater pollution.

Bioassays have a wide variety of uses in pollution control, including the monitoring of pollutant quality and quantity, and the characterization of effluent toxicity. A common practice in bioassays to assess water quality is to use a single test organism. The rainbow trout (Salmo gairdneri) is the dominant freshwater species used by Environment

Canada (21). (It has also been used to test polluted marine waters.) No particular specific marine organism has been selected for bioassays although many bivalves have been used.

Bioassays may be used to measure acute (lethal) toxicity or chronic effects of pollutants on the test organisms. Acute or lethal toxicity is measured as LC_{50} or LD_{50} (LC = lethal concentration, LD = lethal dose). The term refers to the level of a measurable lethal agent required to kill the 50th percentile in a group of test organisms, in a given period of time (21). Usually the time is 96 hours (96 hr. LC_{50}) but it may be 48 or 168 (21). The 50th percentile is meant to represent the average organism. LT_{50} refers to the time it takes for a pollutant or mixture of pollutants to kill the 50th percentile, within 96 hours or other predetermined time.

The most commonly used method for LC_{50}, LD_{50} or LT_{50} determinations are static bioassays, in which the test organisms are in the same or static, measured volume of medium for the duration of the study. The standard procedure for these sorts of tests is given in the Laboratory Manual of Environment Canada, Environmental Protection Service (21).

Continuous flow-through methods are frequently used for sub-lethal contamination assays and may also be used for lethal toxicity tests. In these assays the test organisms are placed in a flowing stream of seawater, which is a closer approximation to their natural environment than is a static volume. The pollutant is added at a know dilution rate, and the effects on the test organism are monitored over a usually lengthy period. Precautions must be taken that contaminants don't enter the system, and that volatile pollutants aren't lost from the system. The flow-through system is essential for long-term studies of chronic, low-level pollution.

Adult bivalves don't lend themselves to short-term, acute toxicity tests, because of their known capacity to accumulate many pollutants at concentrations thousands of times above ambient levels. However, their more fragile life stages, e.g. eggs, embryos and larvae,

have been used successfully as bioassay organisms. Bivalves are also frequently used to determine chronic effects of sub-lethal pollutant concentrations.

5.1 Analyses - Heavy Metals (from EPS lab manual, Dept. of Environment)

5.1.1 Sample Preservation. The recommended technique for all biota is freezing of samples. Cycles of freezing and thawing must be avoided to prevent loss of interstitial or intercellular fluids. Before metal analysis takes place, the tissue sample must be in solution, and a number of preparatory steps may be required, depending upon the tissue itself, and the information desired.

In some cases analyses of specific organs are desired, requiring aseptic dissection. The tissue is then ground, blended or homogenized in various ways, with each step offering opportunities for contamination, unless great care is taken. The tissue is usually then decomposed by one of several methods, and brought into solution for analysis.

5.1.2 Decomposition. Decomposition methods include:

(1) dry ashing - heating in a muffle furnace at 400-800°C in air. Many elements may be lost with this technique and it is not recommended for "extreme trace analysis".

(2) wet ashing - involves liquid reagents and relatively low temperatures so that "losses through volatilization, adsorption and reaction with vessel materials are limited to only a few elements." The wet ashing technique, and reagents employed, depends upon the metals of interest to be analyzed. A list of wet ashing procedures is given in the EPS Laboratory Manual and references to other techniques.

(3) "Low temperature ashing" is considered the "best condition for extreme trace analysis." Samples are decomposed by treatment with an oxygen bearing plasma at temperatures between 100 and 125°C. Besides low temperature, the method has the added advantage of completely destroying the organic material. In many cases the residue can be dissolved in water and a little acid, reducing interference and contamination from other reagents. "Elements which may still be volatilized are Ag, Au, Hg and Se."

5.1.3 Separation and Concentration. "The ideal separation method should offer a large distribution coefficient, rapid equilibrium, minimal interferences at the phase boundary, and low blank values", states the EPS Laboratory manual. Several methods are described, including volatilization techniques, ion exchange methods, and liquid-liquid extraction. The last is commonly used, and the most popular system is MIBK-APCD (methyl isobutyl ketone- ammonium pyrrolidene dithiocarbamate) with atomic absorption spectorphotometry (AAS). The popularity of MIBK as a solvent is attributed to its combustion properties, since it is not especially impressive as an extractant. Gas and liquid chromatography are also used, particularly when organic compounds are being analyzed as well.

5.1.4 Instrumental Methods for Analysis. In most cases analyses rely upon comparison with analyzed standards, many of which are available from the US National Bureau of Standards, e.g. oyster tissue (196). Other calibration methods which can be used are described in the EPS Manual. Of several analytical methods, atomic absorption spectrophotometry (AAS) is the most commonly used, though not without certain pitfalls, which are grouped as (1) spectral, (2) chemical and (3) matrix.

(1) Spectral - refers to element absorption interferences, particularly in the UV. Methods to cope with element and line interference are described in the manual.

(2) Chemical - refers to oxides and other compounds which can interfere with atomic absorption of the desired metals.

(3) Matrix - refers to effects such as viscosity, acid content and surface tension of the matrix which can interfere.

Atomic Emmission Spectrophotometry (AES) or Flame Emission (FE) is not used as commonly as AAS, but is considered to be "highly compatible and complementary" to AAS (21).

Inductively Coupled Argon Plasma (ICAP) is a comparitively new and sophisticated excitation source, which can replace chemical flames. Combined with a computer controlled Optical Emission Spectrometer (OES) "the inherent advantages of AAS have been greatly enhanced" (21). Advantages include (1) a much larger range which allows both trace and major elements to be analyzed at the same sample dilution; (2) As many as 50 or more elements (depending on the instrument) may be analyzed in a sample volume of less than 5 ml; (3) Chemical and matrix intereferences are far less than AAS or AES.

5.2 Organochemicals

5.2.1 PCBs (From Radian Corporation methods). The methods of measuring organochlorinated compounds in animal tissue have been adapted from those used for water, as set forth in the Department of Environment Naquadat (197). PCBs are included in analyses for organochlorinated pesticides such as DDT and many others. Instruction for sample preparation and storage are:

1) samples should be collected and frozen immediately in an all glass system or metal container. Frozen samples may be wrapped in clean aluminum foil for storage.

2) Frozen samples are ground and the mixture made homogeneous. In cases where individual organs are needed for analysis, dissection is done aseptically. Plastic or similar material must NOT come into contact with samples.

Reagents must be of pesticide grade and of highest purity. Usually 8 to 10 grams of tissue is needed for extraction, which is carried out in acetonitrile.

The cleaning up procedures and extraction procedures are often modified, and recovery tests were made for the EPA priority pollutants listed in Table 1. The final analysis can be made with a mass spectrometer.

5.2.2 PAH. The most detailed methods related to PAH in bivalves come from work done in B.C., and published in "Monitoring procedure for chemical carcinogens in coastal waters" (142). The methods as outlined were developed from techniques used to measure PAH in foods. Initially the method involved alkaline digestion of the tissue, then partitioning of the hydrocarbons into isooctane. B(a)P was then purified from the isolate with thin layer chromatography. In some cases the PAH is selectively extracted with dimethylsulfoxide (DMSO) and then back-extracted into fresh isoctane. If interfering materials are not present, the DMSO part of the procedure may be eliminated.

The reproducibility and precision of the results range from 3.2% to 8.1% of the mean, with an average of 6.2%. (Precision was determined with radioactive tracer). The sensitivity of fluorimentric determination of B(a)P is 0.1 ug/kg for 25 g sample, better than 10% precision.

Other descriptions of the methods suggest the use of GC and HPLC instead of TLC.